An evaluation on the effects of three different dietary emulsifiers and the use of black soldier fly (Hermetia illucens) larvae oil on young broiler production

by

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Declaration

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Summary

The aim of the study was to investigate the use of black soldier fly (Hermetia *Illucens* larvae) oil and three dietary emulsifiers in broiler diets. The first part of the study determined the production parameters; organ and intestinal parameters, carcass yield and the physical meat quality of broilers. The broilers received five treatments fed in all three phases (starter, grower and finisher). The treatments consisted of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton and Lysoforte (LYS) at 250g/ton. A total of 300 broilers were used for this study and were grouped and assigned to one of the five diets for 32 days. No significant differences in the growth rate, weekly feed intake, feed conversion ratio (FCR), average daily gain (ADG), European production efficiency factor (EPEF), protein efficiency factor (PEF) and liveability where found between the different treatments. These results indicate that LO could perform on the same level as SF and could replace SF in broiler production. This finding is further demonstrated in the ability of broilers to utilise LO without the help of an emulsifier (no improvement were seen with the use of an emulsifier). No significant differences in the broiler carcass yields (dressing percentage, breast component), nor on their physical meat quality (pH of the breast and thigh and on breast colour) were found between the different treatments. This suggests that the use of LO had no negative effect on these parameters when compared to LO. Furthermore, the lack of differences found between LO with EEP, LES and LYS further demonstrates the ability of broilers to utilise LO efficiently. No significant differences in the organ weight and organ to body percentage of the gizzard, liver, spleen and heart of broilers were found between the different treatments. However, the use of LO showed to have an effect on the bursa of Fabricius size. Broilers receiving LO showed heavier bursa of Fabricius weights when compared to SF and LES. An increase in bursal weight in disease free birds is correlated with an improvement in immune cell production. Therefore, the use of LO in the diet of broilers is able to improve the immune system of birds. Furthermore, the bursa weights of all the treatments still fall within the range that is considered as normal. This indicates that none of the emulsifiers used in the study had a negative effect on the bursa weight. None of the emulsifiers and the use of LO had an effect on the pH of the duodenum, ileum and caeca. However, LES had an effect on gizzard and jejunum pH which may be related to its possible effects on digestion retention time in the gizzard causing a decrease in gizzard pH.This in turn could influence the amount of duodenal secretions needed to buffer against the acidic content (increase in pH) when it enters the intestines. Despite these differences, the pH of the gizzard and jejunum for treatment LES still fell within the normal pH range for both these organs. Significant differences were found in the liver lightness (L^*) between LO with EEP and LYS. The differences found could be speculated based on the interaction of emulsifier type and lipid source on lipogenesis in the body. No significant differences in the gizzard scores were found with the use of the different emulsifiers (at standard and double the standard inclusion level) and LO which suggests that none of the treatments contained any substance that could have negatively affected gizzard health. The second part of the study determined the digestibility parameters of broilers for crude protein (CP),

crude lipid (EE), ash and the apparent metabolizable energy (AME) of the diet. In this study, broilers received the same treatments as described in the first part of the study. A total of a hundred and twenty Cobb 500 broiler chickens were used in the trial. The birds were first acclimatized to a standard control diet for four days (adaption period) after which they were individually weighed and randomly assigned to one of the five treatments. None of the emulsifiers and the use of LO had an effect on the coefficient of total tract digestibility (CTTD) of crude protein, crude lipid and ash. This could suggest that the use of EEP, LES, LYS and LO had no negative effect on the utilization of crude protein, crude lipid and ash by broilers. Therefore, all five treatments performed on the same level. However, EEP negatively affected the apparent metabolizable energy (AME); whereas LES and LYS had no effect on AME. This could suggest that LO is able to be utilised efficiently by broilers without the help of an emulsifier and performed better than SF and therefore could improve the performance of broilers. In terms of crude fibre (CF), no significant differences were found between LO and SF indicating that LO performed on the same level as SF and could be utilised in broiler production without having a negative effect on crude fibre utilization. Significant differences were found between LO with EEP and LYS. The use of LYS showed to have improved CF digestibility whereas EEP reduced it. Furthermore, Emulsifier EEP was the only emulsifier that different significantly from SF and was the least effective treatment at improving CF utilization by broilers. This could therefore, suggest that emulsifier type could affect crude fibre utilization.

Overall, the study indicated that the use of emulsifiers with LO in the diet of young broilers may have an effect on organ and intestinal pH, thigh colour, crude fibre and AME digestibility. Whereas, the use of LO have shown to have no side effects on normal broiler production), meat quality traits, gizzard health and on nutrient utilization when compared to SF. This indicates that LO could be seen as a promising alternative to sunflower oil in broiler production. The use of the different emulsifiers in the diet showed no negative effect or improvement on broiler production, organ and intestinal pH but may have had an effect on thigh colour, crude fibre and AME digestibility. Therefore the use of an emulsifier and fat type could have an effect on broiler production parameters.

Opsomming

Die doel van die studie was om ‘n ondersoek te loods na die effek van drie diëet emulsifiseerders en venstervlieg (*Hermetia illucens*) larwe olie as die hoof vet bron op die produkie potensiaal van braaikuikens. Tydens die studie was die produksie parameters; orgaan en intestinale parameters, karkas opbrengs en die fisiese vleiskwaliteit van die braaikuikens bepaal. 'n Totaal van 300 braaikuikens was in hierdie studie gebruik. Die braaikuikens was lukraak in vyf groepe van 60 elk gegroepeer en is vir 32 dae van ‘n spesifieke dieet voorsien. Die diëte het Excential energy plus (EEP) teen 250g/ton, Lesitol (LES) teen 0.2L/ton en Lysoforte (LYS) teen 250g/ton, LO (venstervlieglarwe olie) (positiewe kontrole) en sonneblom olie (SF) (negatiewe kontrole) bevat. Geen beduidende verskille is gevind in die groeikoers, weeklikse voeriname, voeromsettingsverhouding, gemiddelde daaglikste toename, Europese produksie effektiwiteits faktor , proteïen-effektiwiteitsfaktor en op die leefbarheid tussen die verskillende behandelings nie, wat daarop dui dat al die behandelings ewe goed presteer het.. Geen beduidende verskille is gevind in die braaikuiken karkas opbrengste (uitslagpersentasie, bors komponente), of op die fisiese vleiskwaliteit (pH van die bors , dy en borsvleis kleur) vir die verskillende behandelings nie. Dit dui daarop dat die gebruik van die drie verskillende emulsifiseerders en LO geen negatiewe uitwerking op hierdie parameters gehad het nie. Tussen die verskillende behandelings was daar ook geen beduidende verskille in die orgaan gewigte en orgaan tot liggaam verhoudings van die spiermaag, lewer, milt en hart van die braaikuikens gevind nie. Maar, die gebruik van LO het 'n uitwerking op die bursa van Fabricius grootte getoon. Braaikuikens wat die LO ontvang het se bursa was swaarder hoewel die bursa van Fabricus nog steeds binne die normale waarde geval het en die grootte verskil in verhouding tot die finale liggaam gewig en individuele variasie. Nie een van die emulsifiers asook die gebruik van LO het 'n invloed op die pH van die duodenum, ileum en caeca getoon nie. Lesitoldarenteen het 'n uitwerking op die spiermaag en jejunum pH gehad wat kan verband hou met die moontlike uitwerking op die retensietyd in die spiermaag wat weer 'n afname in spiermaag pH kon veroorsaak. Op sy beurt mag dit weer die hoeveelheid duodenale afskeidings beïnvloed wat nodig is om as buffer op te tree teen die suurinhoud (pH). Ten spyte van hierdie verskille, het die pH van die spiermaag en jejunum vir behandeling LES nog binne die normale pH-reeks vir beide hierdie organe geval. Beduidende verskille was gevind in die lewer ligtheids kleur (L*) tussen LO met EEP and LYS. Die verkille wat gevind is, kan bespiegel word op grond van die invloed van emulgatoren op lipogenese in die liggaam. Geen beduidende verskille in die spiermaag klasse is gevind met die gebruik van die verskillende emulsifiers (op standaard en dubbel die standaard insluitingsvlakke) en LO nie. Dit dui daarop dat nie een van die behandelings enige stof bevat wat ‘n negatiewe effek op die spiermaag gesondheid mag hê nie. Die tweede fase van die studie het die verteerbaarheid parameters van braaikuikens vir ru-proteïen, ru vet, as en die skynbare metaboliseerbare energie (SME) van die dieet bepaal. 'n totaal van 120 Cobb 500 braaikuikens is in die proef gebruik. Die kuikens het vir vier dae (die aanpassing tydperk) 'n standard kontrole dieet, gekry waarna hulle in groepe van drie aan een van die vyf behandelings toegedeel is. Die braaikuikens het ‘n drie fase dieet (aanvang, groei en afronding) met vyf behandelings ontvang. Behandelings wassoortgelyk aan die van die eerste proef.

Die mis en oorskiet kos was oor vyf dae versamel, geweeg en in die vrieskas gestoor. Voor ontleding, was elke hok se mis saamgevoeg en in die oond gesit tot dat dit droog was. Daarna is dit fyn gemaal. Nie een van die emulsifiseerders of die gebruik van LO het 'n uitwerking op die totale spysverteringskanaal verteerbaarheid van ru-proteïene, ru-vet of as gehad nie. Dit kan daarop dui dat die gebruik van EEP, LES, LYS en LO geen uitwerking op die benutting van ru-proteïen, ru-vet en as deur braaikuikens het nie. Dit wil sê al vyf behandelings het dieselfde resultate gelewer. Excential energy plus het egter 'n negatiewe uitwerking op die die SME gehad; terwyl LES en LYS geen effek op AME gehad het nie. Dit kan dui daarop dat LO in staat is om doeltreffend deur braaikuikens benut te word sonder die hulp van 'n emulsifier en beter presteer as SF en dus die produksie effektiwiteit van braaikuikens kan verbeter. In terme van ru vesel, het LES gelei tot die hoogste ruvesel verteerbaarheid; terwyl EEP gelei het tot die laagste ruvesel verteerbaarehid, terwyl LYS ‘n effense verbetering in ruvesel verteerbaarheid gehad in vergelyking met LO. Dit kan daarop dui dat die tipe emulsifiseerder ruvesel verteerbaarheid kan beïnvloed. Die gebrek aan verskille wat tussen LO en SF voorkom, dui daarop dat LO op dieselfde vlak as SF gebruik kan word in braaikuiken produksie sonder om 'n negatiewe uitwerking op ruveselbenutting te hê. In die algemeen dui die studie daarop dat die gebruik van emulsifier met LO in die dieet van jong braaikuikens wisselende uitwerkings op orgaan en derm pH, dyvleiskleur, verteerbaarheid van ruvesel en SME het. Die gebruik van LO het geen nadelige effekte op normale braaikuiken produksie, vleis kwaliteitseienskappe, spiermaaggesondheid of verteerbaarheid in vergelyking met SF getoon het nie. Dit dui daarop dat LO gesien kan word as 'n belowende alternatief vir sonneblomolie in braaikuiken produksie.

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Notes

The language and style used in this thesis are in accordance with the requirements of the South African Journal of Animal Science. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

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Abbreviations

CP	Crude protein
CTTD	Coefficient of total tract digestibilty
DFD	Dark, fir and dry
DHA	Docosahexaenoic acid
DM	Dry matter
EE	Ether extract
EEP	Excential energy plus
EPA	Eicosapentaenoic acid
EPEF	European Production Efficiency Factor
EU	European Union
FAS	Fatty acid synthase
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
g	Gram
GE	Gross energy
GIT	Gatsro-intestinal tract
HCL	Hydrochloric acid
HETrR	Hydroxyl-eicosatrienoic acid
HLB	Hydrophilic-lipophilic balance
IBVD	Infected bursal disease virus
J	Joules
Kg	Kilogram
L	Litres
L^*	Lightness
LA	Linoleic acid
LES	Lesitol
LO	Black soldier fly larave oil
LPC	Lysophosphatidycholine
LYS	Lysoforte
m	Meters
ME	Metabolizable energy
min	Minutes
MJ	Mega joules
n-3	Omega 3
n-6	Omega 6
NE	Necrotic enteritis
pHi	Initial pH
pHu	Ultimate pH
PSE	Pale Soft Excudate

SAPA	South African Poultry Association
SF	Sunflower oil
SFA	Saturtaed fatty acids
SSL	Sodium stearoyl-2-lactylate
UFA	Unsaturtaed fatty acids

Chapter 1
General Introduction

With an increase in the world population there is also an increase in the demand for animal protein sources (Čičková *et al.*, 2015). In South Africa, poultry meat was the most consumed animal protein source followed by beef, pork, mutton and goat with the exception of milk (SAPA, 2017). Demand in poultry meat is attributed to various factors of which include an increase in income, increases in pork and beef prices and increases in the preference for poultry meat (Narrod *et al.*, 2008). The increased preference for poultry meat is mostly due to its nutritional aspects (Narrod *et al.*, 2008). Poultry meat is a low-lipid animal protein, contains a high proportion of unsaturated fatty acids and contains low levels of sodium and cholesterol (Petracci & Cavani, 2012). The high growth rate and feed efficiency are the two main targets in broiler production (Sugiharto, 2016). Therefore, the most important key in the broiler industry is to provide feed that contains all the necessary nutrients needed for broilers to grow to their full genetic potential (Cullere *et al.*, 2016). Unfortunately, the greatest expenditure in broiler production is feed cost that can make up to 60-75% of the total cost (Ding *et al*., 2016). The use of supplemental fats (animal or vegetable) in the diets of poultry may be seen as a cheaper way to increase the energy value of the diet in order to meet the energy requirements of birds (Doreau & Chilliard, 1997) due to fats have a higher energy value and can provide about 2.25 times more energy than carbohydrates (Lauridsen *et al.*, 2007).

Unfortunately, one of the biggest challenges the poultry industry is facing is the physiological limitation of young broilers to utilise certain fats effectively as an energy source due to the lack of many digestive enzymes (Ding *et al.*, 2016). These physiological limitations are attributed to young broilers during the first two weeks post hatch having poor bile salt production and thus inadequate amounts of bile salt needed for effective lipid digestion (emulsification) (Siyal *et al.*, 2017a). The first two weeks of a chicks life *post*-hatch represents 20% of the chicks production cycle (Tancharoenrat *et al.*, 2013) and without efficient lipid digestion the production ability of chick can be negatively affected (Siyal *et al.*, 2017a). The addition of an emulsifier to the diets of young broilers may be a way to help improve lipid digestion and thus utilization of fats without having a negative effect on the performance of broilers (Abbas *et al.*, 2016). The main action of dietary emulsifiers is to increase the active surface area of fats thereby enhancing the action of lipase for the hydrolysis of lipids into triglycerides and monoglycerides for the formation of micelles (Upadhaya *et al.*, 2018). Micelles are formed as a product of lipid digestion and therefore consists of bile salts, fatty acids and monoglycerides (Leeson, 1991). Saturated fatty acids require efficient emulsification by bile salts to form micelles; whereas unsaturated fatty acids are able to spontaneously form micelles (Ravindran *et al.*, 2016). Therefore, the supplementation of emulsifiers in the diet of young broilers could help to incorporate fatty acids into the micellar phase (Dierick & Decuypere, 2004) by assisting in lipid digestion during the stages of limited lipid digestion by broilers.

Most of the studies on emulsifier used commercial oils of which include, sunflower oil (Zampiga *et al.*, 2016), soybean oil (Boontiam *et al.*, 2017) tallow (Upadhaya *et al.*, 2018) and palm oil (Aguilar *et al.*, 2013). There is a lack of research on the use of emulsifiers with black soldier fly larvae oil. One of the benefits in the utilization of insects in the feed of birds is that insects naturally form part of the diet of free-range poultry (Cullere *et al.*, 2016). Insect species used as feed resources can be grown in a sustainable manner (usually in warehouses) which requires less land usage and water when compared to crops (Sánchez-Muros *et al.*, 2014). Black soldier fly (*Hermetia illucens*) belongs to the Stratiomyidae family and is indigenous to the tropical, subtropical and warm areas of America (Makkar *et al.*, 2014). The larvae of this fly have the ability to accumulate large amount of lipids in their body, when provided with the appropriate lipid-rich diet, and when used as a lipid source in the diet of animals, the larvae were more palatable compared to fish and vegetable oils (Wang & Shelomi, 2017). The use of black soldier fly larvae oil has shown to yield positive results and include improvement in amino acid digestion and better utilization of the energy of the diet (Schiavone *et al.*, 2017a; b).

Therefore, the aim of the study was to investigate the efficacy of three emulsifiers, black soldier fly oil and sunflower oil on broiler production parameters; organ-and intestinal parameter; carcass and meat quality characteristics and total tract digestibility.

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Chapter 2

Literature Review

2.1 Introduction

Since 1970, the global consumption of poultry products such as meat and eggs has increased significantly (Yegani & Korver, 2008). This growth has been attributed to various factors such as an increase in the household income, stronger preference for poultry meat and the economic reality that poultry is a cheaper source of protein than other protein sources from animal origin (Narrod *et al.*, 2008). The ability of the poultry industry to keep up with this demand is due to the rapid improvement in their production practices such as management, health, nutrition and genetics (Vieira & Angel, 2012) as well as increases in their processed meat production (Hoffmann, 2019). However, one of the major challenges the poultry industry is facing is the physiological inability of young birds to utilise fats and oils effectively. A challenge exists in providing a cost-effective diet that can be utilized effectively by young birds to provide them with the needed energy requirement for efficient growth. Young birds are not able to effectively utilize fats and oils as effectively during the first few weeks *post*-hatch due to their immature digestive system (Yegani & Korver, 2008) which can lead to production and economic strain. According to Tancharoenrat *et al*. (2013), the first week of a chicks life can account for 20% of the total grow-out period which is significant from a production perspective. However, during this early stage of a chick's life their digestive system has not fully matured and therefore cannot produce enough bile nor can recirculate the small amounts that are secreted (Siyal *et al.*, 2017a). Bile is a natural emulsifier found in the digestive tract of animals and is responsible for the breakdown of fats into lipid droplets and the activation of lipase for further lipid digestion (Ravindran *et al.*, 2016). Synthetic bile can be used to help aid in lipid digestion but the use of this product is too costly and can result in high production costs (Khonyoung *et al.*, 2015).The use of emulsifiers in the poultry industry is considered a new practice when compared to other feed additives such as antibiotics, that have been used for decades (Neto *et al.*, 2011). Emulsifiers can be seen as a means to help aid young birds in the digestion of fats and thereby taking full advantage of their growth potential. The use of other emulsifiers is a much better option in that it can ensure that a lower energy diet that is more economical can still provide enough energy to maintain the energy requirements of high performance broiler strains (Siyal *et al.*, 2017b). Emulsifiers are unique in their ability to act as a catalyst in lipid digestion. They are able to increase the surface area of lipid globules and in doing so also enhances the hydrolytic action of lipase to produce fatty acids and monoglycerides (Upadhaya *et al.*, 2018). This is a vital step for effective lipid absorption as the by-products of lipid digestion (fatty acids and monoglycerides) are needed for the formation of micelles (Zhang *et al.*, 2011). Moreover, it is only in the micelle formation whereby lipids can be effectively absorbed across the lumen in the small intestine. Therefore, the use of emulsifiers in the diet of broilers could be considered an alternative method to bile salts to aid in the efficient digestion of feed by young broilers.

2.2 Importance of energy for growth in young broilers

Over the past few years the result of genetic development in modern broiler strains for increased growth, has led to an increase in their energy requirements (Tancharoenrat *et al.*, 2013). Energy constitutes one of the major cost components in feed formulations (Siyal et al., 2017a) and therefore nutritionist have focused their attention more and more on the use of fats and oils. Fats and oils are used in the diet of broilers as a high-energy ingredient (Azman *et al.*, 2004) to provide efficient amount of energy for optimal functioning (growth and maintenance) (Tancharoenrat, 2012). Dietary energy is used for various biological processes but in production animals, such as broilers, the focus on energy is mainly for the energy available for the production of meat. Although energy is not a nutrient *per se* it is one of the most important factors in any production chain. Nutrients such as fats, carbohydrates and proteins are digested in the digestive tract of poultry and are then absorbed across the intestinal lumen to be metabolized in the cells for energy. To determine the amount of energy available for production, the following needs to be understood: (1) the composition of the dietary feed and how well it can be digested as this directly influences the amount of energy that can be available to the bird and (2) how much of the dietary energy is used for maintenance (Bolton & Blair, 1974).The unit of measure for energy is Joule (J) but has also been expressed as calories (Ca) but in terms of the energy requirements of the birds, it is expressed more as the metabilizable energy (MJ/kg of feed) that is needed per animal per day to ensure for normal growth and development (Scott *et al.*, 1976). One of the most important aspects in the understanding of dietary energy is its effects on feed intake. “The primary response to dietary energy concentration is seen in feed consumption and in productive efficiency rather than in production level” (Classen, 2016). This statement implies that a bird will continue to consume feed to meet its energy demands; therefore the dietary energy can be considered the driving force effecting feed intake as long as all the other nutrients are provided at the correct levels.

2.3 Fats and oils (Lipids)

Fats and oils are common ingredients (Classen, 2016) that has been used for years by nutritionists as an energy source to increase the energy density of poultry diets (Ravindran *et al.*, 2016). The increasing interest in fats and oils as the main energy source is due to the increase in the price of traditional energy sources from cereals such as maize (Baião & Lara, 2005). In animal nutrition, fats and oils are termed lipids and are one of the most important components in the diet of broilers (Doreau & Chilliard, 1997). The use of fats in the diet of broilers provides many advantages. These advantages include improved reabsorption of liposoluble vitamins, increased retention time which allows for better utilization of the feed, provides essential fatty acids and acts as a concentrated source of stored energy (Baltic *et al.*, 2017). Other benefits include improved texture of the feed provided, reduced dustiness and an increase in the consumption of food due to increased palatability (Tancharoenrat *et al.*, 2013).

Physiologically, the use of proteins and amino acids as the main energy source is not considered practical as these components are more expensive (economically) to be used as an energy source

when compared to lipids. In other words, more energy is wasted to convert proteins into glucose and places too much metabolic strain on the animal (cost of deamination and the increase in uric acid production and the elimination of it) (Scott *et al.*, 1976). Moreover, the energy produced from lipids is 2.25 times more than the energy produced from starch in that the carbon of lipids are chemically more reduced than the carbon found in sugars (Baião & Lara, 2005). In other words, 37.7J can be extracted from one gram of lipid (Goenewald & Boyazoglu, 1980) as opposed to 16.7J from one gram of carbohydrates (Craig & Helfrich, 2017).

2.3.1. Different types of fats used in the poultry industry

There is a diverse range of lipids sources that can be utilised in the feed of poultry but the most common include restaurant grease, rendering by-products, vegetable oils and acidulated soap stock and their selection is highly influenced by cost and availability (Ravindran *et al.*, 2016). However, not all fats can be effectively digested by young birds when compared to older birds (Siyal *et al.*, 2017b). Tancharoenrat *et al.*, (2013) showed that even though chicks could not digest unsaturated and saturated fats as effectively during their first week post-*hatch*, the proportion of fats containing high concentration of saturated fatty acids (SFA) were digested more poorly than those fats containing high unsaturated fatty acids (UFA). This is in part due to the action of lipase and its preference to hydrolyse lipids at the sn-1 and sn-3 positions (Ravindran *et al.*, 2016). In stereochemical terms, the structure of fats and its acid chains are numbered according to their positions, for example sn-1, sn-2 and sn-3 on the glycerol backbone. Most fats of animal origin are high in fatty acids in the sn-1 and sn-3 positions (Meng *et al.*, 2004) and are not as effectively absorbed as those fatty acids on the sn-2 position. Saturated fats such as beef tallow have about 73 to 81% of its saturated fatty acids palmitic and stearic acids at the sn-1 and sn-3 positions (Sibbald *et al.*, 1961) making it less digestible by young birds. Fatty acids at the sn-2 position are more readily absorbed due to its amphilic properties and also acts as a natural emulsifier improving fatty acid digestion and absorption (Smink *et al.*, 2004). Therefore, when providing fats as an energy source into the diet of young broilers, fat type plays an important role as it affects the ability of young birds to utilize the dietary fat as an energy source. Another important factor to consider when selecting lipid sources are that, when lipids absorbed and transported, lipids undergo little to no alterations therefore importance should also be placed on the utilization of safe lipids that have a high nutritional value (van Ruth *et al.*, 2010) as there is a similarity between the lipid provided and body lipid that is deposited (Baião & Lara, 2005).

2.3.2 Different classes of lipids

Lipids, whether they be fats (solid at room temperature) or oils (liquids at room temperature) are esters of glycerol and fatty acids and can be divided into three groups and include simple, compound and derived lipids. Simple lipids consists of esters of fatty acids and certain alcohols particularly glycerol and cholesterol. Esters containing an alcohol, other than glycerol, are called waxes. Compound lipids consist of esters of glycerol with two fatty acids and another chemical group. Examples include phospholipid lecithin, cephalin, choline and sphingomyelin. Derived lipids include substances that have been produced by the hydrolysis of simple lipids and compound lipids. These

include fatty acids, alcohols (glycerols, cetanol and lanol) and sterols (cholesterol, sitosterol and ergosterol) (Scott *et al.*, 1976).

2.3.3 Fatty Acids

Fatty acid, are components formed through the hydrolysis of fats or oils (Zhang *et al.*, 2011) and chemically consists of carbon, hydrogen and oxygen that are arranged to form a carbon chain that contains a carboxyl (COOH) group at one end with a methyl (CH3) group situated at the other end (Cherian, 2011). Fatty acids can be grouped into either saturated (SFA) or unsaturated fatty acids (UFA). Both components contain carbon, hydrogen and oxygen but the difference lies in the number of bonds present in the carbon chain. Saturated fatty acids contain single carbon bonds in the carbon chain whereas UFA contains double carbon bonds. Fatty acids that contain more than one double bond are termed polyunsaturated fatty acids (PUFA) whereas those containing only one double bond are termed monounsaturated fatty acids (MUFA). Another difference between the fatty acid classes is the way they interact with atmospheric oxygen. Due to the makeup of SFA, they are more resistant to oxidation when in contact with atmospheric oxygen whereas UFA are more prone to oxidation due to the degree of saturation (Bolton & Blair, 1974). All dietary fatty acids whether that SFA or UFA have approximately the same amount of energy per unit of mass (Hulbert & Abbott, 2011). Fatty acids play a major role in the health of poultry. These include the synthesis of membranes, alterations to carbohydrates and proteins, the ability to solubilize a number of nonpolar and poorly soluble cellular and extracellular components, their aid in the building of structural elements in cells and tissues, the development of signalling compounds and lastly to act as a source of energy (German & Dillard, 2004). Importantly, fatty acids also play a major role in the formation of micelles thereby controlling the efficiency of lipid absorption (Zhang *et al.*, 2011).For instance, monoglycerides play a vital role in the absorption of many fatty acids. The molecular structure of monoglycerides allows it to act as a natural emulsifier (contains a hydrophilic and a hydrophobic component) and therefore aids in the incorporation of fatty acids into the micellar phase (Ravindran *et al.*, 2016). Therefore, it's important that young broilers are able to digest lipid sources effectively due to the various importances of different fatty acids for optimal functioning.

2.3.3.1 Essential Fatty acid

Fatty acids are considered essential when they cannot be synthesized within the body and without sufficient amounts it can lead to impaired physiological functioning (Forbes & Parsons, 2012). For example, during the early stages of a hatchlings life these metabolic precursors play a vital role for proper growth development and deficiencies can lead to growth impairment (Cherian, 2015). In poultry nutrition, α-linolenic acid (ALA 18:3 n-3) and linoleic acid (LA 18:2 n-6), are part of the omega-3 and omega-6 fatty acid groups respectively and are considered as essential fatty acids (Cherian, 2015) . Both these fatty acids contain double bonds at the 18th carbon position (Forbes & Parsons, 2012). Importantly these fatty acids play an important role in the production of eicosanoids such as prostaglandins and leukotrienes that are involved during the inflammatory process (a defence mechanism to protect the bird against infections) (Cherian, 2015) . Linoleic and linolenic acids also play an important role in the provision/formation of very important C20 acids namely eicosapentaenoic (EPA) ,hydroxyl-eicosatrienoic (HETrR) and docosahexaenoic (DHA) acids that are all involved in the

fluidity of mammalian cell membranes (McDonald *et al.*, 2011). Eicosapentaenoic acids play an important role in the anti-inflammatory process and the inhibition of the platelet processes, DHA is thought to have a significant role in the functioning of the brain as well as retinal functioning whilst HETrR plays an important role in modulating the effects of eicosanoids production from arachidonic acid (McDonald *et al.*, 2011).The absence of essential fatty acids in the diet of poultry can confer serious health problems. These include the reduction of egg size in laying hens (Bolton & Blair, 1974), weakening of the immune system (increased susceptibility to diseases), growth impairment (retarded growth in young birds), decrease in testes development and in the development of secondary of sexual characteristics in males (Ravindran *et al.*, 2016).

2.4 Sustainable lipid sources

The utilisation of lipids in the diet of poultry is a widespread practice, however more nutritionists are researching various quality lipid sources with more competitive prices (Vilarrasa *et al.*, 2015). The increase in the world population has forced the increase development of intensive animal feeding systems in order to meet the growing demand for animal proteins (Čičková *et al.*, 2015). Unfortunately, this has led to the overexploitation of natural feed resources as well as the increase in the demand and price of conventional feedstuff which has forced nutritionists to seek more sustainable and environmentally friendly resources to be used in animal feed (Cullere *et al.*, 2016). In other words, one of the biggest challenges the livestock industry is currently facing is to produce enough animal protein products to meet the protein demands of the world but at the same time to reduce the negative environmental footprint of production (Hume *et al.*, 2011). One very promising alternative is the utilization of insects as a sustainable feed sources for livestock such as poultry (Cullere *et al.*, 2016).

2.4.1 The use of black soldier fly as an alternative lipid source

One of the benefits in the utilization of insects in the feed of birds is that insects naturally form part of the diet of free-range poultry (Cullere *et al.*, 2016). Insect species used as feed sources can be grown in a sustainable manner (usually in warehouses) which requires less land usage and water when compared to crops (Sánchez-Muros *et al.*, 2014). Not only can insects be used as a feed source for poultry, they can also utilize organic waste such as animal manure (Sheppard *et al.*, 1994) and food waste (Rehman *et al.*, 2017) as a feed source thereby greatly reducing the negative impact that waste can have on the environment (Wang & Shelomi, 2017). There are various types of insects that have been studied as a feed source for animals and include, for example, mealworm larvae, housefly larvae and pupae, locusts, crickets, grasshoppers, silkworm and black soldier fly (Makkar *et al.*, 2014). Over the past few years, black solider fly (BSF) has been identified as one of the most promising insect species for use in the livestock industry especially in broiler production (Schiavone *et al.*, 2017b).

Black soldier fly (*H. illucens*) belongs to the Stratiomyidae family and is indigenous to the tropical and subtropical and warm areas of America (Makkar *et al.*, 2014). A major advantage in the use of black soldier fly when compared to other insect species that are used for biomass production is that adult

flies do not require feed and thus do not require any particular care (Makkar *et al.*, 2014). Black soldier fly are not considered as pets (Liu *et al.*, 2017) due to their preference for vegetation and their lack of interest to approach animals and humans (Čičková *et al.*, 2015). When provided with the ideal growing conditions at around 25-30 °C together with plentiful supply of food, the larvae of this fly can grow and develop into prepupae within two weeks but three to four weeks is not uncommon (Lalander *et al.*, 2013). The larvae of this fly have the ability to accumulate large amount of lipids in their body, when provided with the appropriate lipid-rich diet, and when used as a source of lipid in the diet of animals, BSF larvae were more palatable compared to fish and vegetable oils (Wang & Shelomi, 2017). Nutritionally, the larvae of this fly species contain more crude protein and lipids compared to the housefly (*Musca domestica*), has a high ash content and has a dry matter from 11 to 28% (Makkar *et al.*, 2014). The lipid content of the pre-pupae of this fly species is 118 g/kg, dry matter (DM) with a protein content of 476 g/kg (Kroeckel *et al.*, 2012) making it a promising substitute for soybean meal. The use of BSF in broiler production has shown to yield positive results. For instance, the supplementation of BSF in the diet of broilers had no effect on the sensory, cholesterol and oxidative status of the meat (Wang & Shelomi, 2017). Uushona (2015) also found that the supplementation of BSF had no effect on the sensory attributes for chicken aroma and flavour. In fact, the supplementation of BSF improved the nutritional value of the broiler meat by increasing the threonine, tyrosine, aspartic, serine, alanine and glutamic acid levels (Wang & Shelomi, 2017). Therefore, BSF could be used as promising alternative and sustainable lipid source in broiler production.

2.4.1.1 Other uses of black soldier fly

As the human population continues to increase, there is also an increase in animal waste, residential waste, commercial waste and institutional waste all of which can cause major environmental pollution and health hazards (Li *et al.*, 2011). With the increased demand in animal protein, there is also an increase in the amounts of manure produced by large farms and other agricultural wastes but unfortunately there is not enough land available for proper waste disposal (Čičková *et al.*, 2015). Furthermore, food wastage is also becoming more and more a significant problem and there is an urgent need to find and develop competitive ways to recycle food wastage in an efficient sustainable manner (de Cossío *et al.*, 2017). Black soldier fly larvae are considered efficient bio-converters in that they can utilize a variety of decaying matter including rotting fruits and plant residues as a feed substrate (Čičková *et al.*, 2015). They also have the ability to convert fresh manure into compost (Webster *et al.*, 2016). Furthermore, when using these organic waste as a substrate, they are able to restrict bacterial growth within the waste reducing the production of obnoxious odours (Makkar *et al.*, 2014). Another benefit in the use of BSF larvae as bio-converters of manure is that they make manure more fluid like; a state that is not favourable for the house fly (*M. domestica*) and BSF larvae has been shown to reduce housefly populations of pig and poultry manure by 94-100% (Makkar *et al.*, 2014). Other use of BSF in manure management benefits includes the reduction of manure bulk by half or more in comparison to unoccupied manure as well as the ability of these larvae to produce nutritious larvae feedstuff that is economically attractive (Sheppard *et al.*, 1994).Nguyen *et al.* (2015) focused on the impact larval diet would have on the development of black soldier fly larvae. The study

focused on six types of waste which included a standard poultry feed, pig liver, pig manure, kitchen waste, fruits and vegetables and fish rendering. It was concluded that BSF could consume and reduce all waste types but kitchen waste showed the greatest mean reduction rate (consumption per day) and produced BSF larvae that were the longest and heaviest. Liver, fruits and vegetables, manure and fish resulted in BSF larvae that were the same length and weight as those reared on poultry feed. Therefore, the ability of BSF to utilize various waste and to produce nutritious larvae emphasis its dual sustainable and economic aspects that should be taken advantage of in the livestock industry.

2.5 The digestive tract of poultry

The digestive system of poultry is made up of the mouth, crop, proventriculus, gizzard, duodenum, small intestine, large intestine, ceca and the cloaca (Bolton & Blair, 1974) (Figure 2.1). Each organ plays a unique role in the ingestion, digestion, absorption and excretion of the consumed feed. The oral cavity in birds consists of a beak and a tongue but teeth and lips are absent. The function of the beak is to scoop up food whereas the tongue is needed for directing and pushing food down into the oesophagus (Moreng & Avens, 1985). The crop is a pear shape sac that is located at the diverticulum of the oesophagus (McDonald *et al.*, 2011). Its main function is to provide storage and the moistening of the feed in preparation for mixing and digestion in the gizzard and proventriculus (Moreng & Avens, 1985). The gizzard is known as the muscular stomach and is responsible for the physical breakdown of large particles through the rhythmic grinding motions of its muscles, whereas the proventriculus is known as the ‘glandular stomach’ and is the first site where digestive enzymes are secreted for the start of significant lipid digestion (Moreng & Avens, 1985). The small intestine consists of the duodenum, jejunum and the ileum. The duodenum is the site were the digesta and secretions mix (McDonald *et al.*, 2011). The jejunum is the middle segment of the small intestine and plays a key role as most of the major nutrients are digested and absorbed here. The ileum is the last segment of the small intestine and its main role is believed to act as the site of water and mineral absorption and to a smaller degree the digestion and absorption of starch (Svihus, 2014). The large intestine in poultry consists of a pair of caeca that are joined to the rectum and continues into the colon and cloaca. One of the main functions of the large intestine is the breakdown of small amounts of undigested fibre from the diet (Moreng & Avens, 1985) as well as the absorption of water and electrolytes (Svihus, 2014). Lastly, the cloaca is responsible for the excretion of faeces and urine (McDonald *et al.*, 2011).

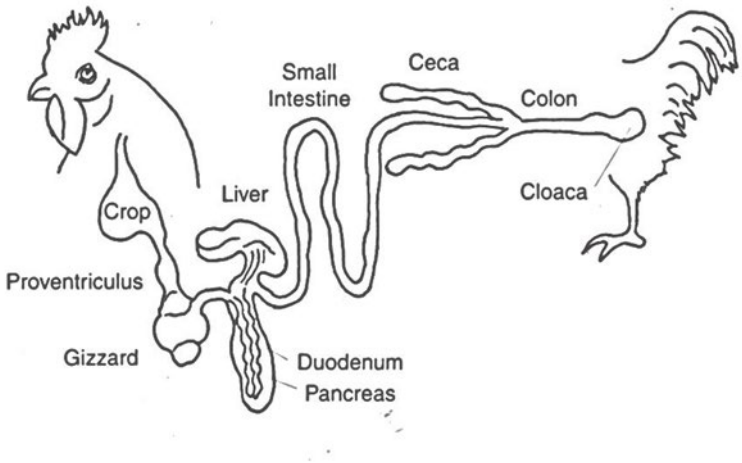

Figure 2.1 Schematic of avian digestive system (Leeson & Summers, 2005)

2.5.1 Lipid digestion

The main site of significant lipid digestion in poultry is within the aqueous environment of the small intestine (Zhang *et al.*, 2011). Fats cannot be absorbed in its whole state and thus needs to undergo lipid hydrolysis before it can be effectively absorbed. Bile and pancreatic lipase are enzyme excretions that are mainly responsible for the mediating of lipid hydrolysis in the small intestine. Bile is a green-coloured liquid made up of bile salts and a lipase accelerator (co-lipase) that is stored in the gall bladder and is secreted by the liver into the duodenum for lipid emulsification (McDonald *et al.*, 2011). During lipid emulsification, bile salts together with phospholipids actively break down lipid globules into smaller lipid droplets thereby increasing the surface area to enhance the catalytic ability of lipase (Khonyoung *et al.*, 2015). The pancreas is responsible for the secretion of lipase and co-lipase. Co-lipase is an important protein needed in enhancing the actions of lipase during lipolysis (Krogdahl, 1985). Co-lipase contains both hydrophobic and hydrophilic amino acids and when it reacts with lipase it allows for the active configuration of lipase to be maintained at the lipid-water interface (Ravindran *et al.*, 2016). By maintaining this active configuration lipase is then able to reach its substrate. The products produced by lipolysis (fatty acids, glycerol and monoglycerides) are then combined to form micelles (Figure 2.2). Micelles are therefore aggregated molecules consisting of lipid molecules (products of lipid hydrolysis) of polar and nonpolar groups that are arranged so that the polar groups are exposed on the outside (in contact with the aqueous environment) and the nonpolar groups form the inner core (Krogdahl, 1985). Not only does micelles control lipid absorption but also plays an important role in the solubilizing and absorption of high amounts of fat-soluble vitamins (Baião & Lara, 2005).

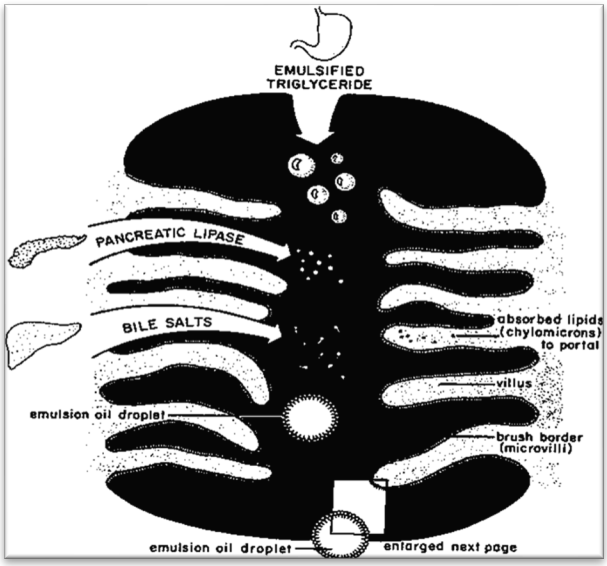

Figure 2.2 Schematic depiction of the initial stages of lipiddigestion in the duodenum (Scott et al., 1976)

2.5.2 Lipid absorption

Micelle formation greatly influences how effective fats are absorbed and therefore controls how well the products of lipolysis are absorbed across the small intestine (Dierick & Decuypere, 2004).The process of micelle formation is not well understood on a chemical level but physiologically it can be understood on a simpler level. The products of lipolysis are absorbed differently across the small intestinal lumen. Those compounds that readily form micelles are passively absorbed from the lumen into the mucosal cell membrane (Leeson & Summers, 2001). Those compounds that cannot readily form micelles such as amphiphilic compounds (monoglycerides, unsaturated long-chain fatty acids and medium-chain fatty acids) form micelles with bile salts to form a lipid-soluble liquid crystal that has the ability to then solubilize other compounds such as fat-soluble vitamins and cholesterol esters (Krogdahl, 2016). The formation of micelles of amphiphiles is an important step that is needed for the absorption of other non-soluble compounds and therefore acts as a medium in which these compounds can then be absorbed into the lumen.

Leeson & Summers (2001) provides in-depth information on the process from when the micelles enter the mucosal cell membrane and the transportation of these micelles within the system. Briefly, once the micelles enter the mucosal cell membrane, the compounds of the micelles are re-esterified (triglycerides). The triglycerides together with free fatty acids, lipoproteins and phospholipids combine to form into a compound know as a chylomicron. Chylomicrons structurally consist of a core of the re-esterified triglycerides surrounded by a membrane like structure consisting of protein, cholesterol and phospholipids. It is in this form that the re-esterified triglycerides are transported into the systemic circulatory system of the body. Lipids that are absorbed from the intestinal lumen of the bird undergo little to no alterations in their fatty acid composition thus there is a significant similarity in the dietary lipid provided to the bird and body lipid that is deposited (Baião & Lara, 2005).

2.5.3 The physiological inability of young broilers to digest fat

The post-hatch chick faces many challenges of which one is the dietary adaptation from utilizing yolk as the main dietary source during embryonic development to the utilization of complex dietary nutrients within a short period of time *post-hatch*. Their small intestine is still immature and during the first two weeks of life the intestines undergo many morphological and biochemical changes (Yegani & Korver, 2008). Initially, during embryonic development, chicks are completely dependent on yolk lipids as well as some residual yolk lipids that is still available shortly after hatching (Sell, 1996). Yolk present in the small intestine of the chick contains its own factors needed for digestion and absorption. These factors needed for digestion and absorption is brought about by lipases that originate from the internal surface of the yolk sac thus the use of lipase from the pancreas and biliary secretions is not needed until the chick starts to consume feed (Krogdahl, 1985). The inability of the immature small intestine to utilize feed effectively during the first few weeks' post-*hatch* can have a great impact on their anatomical development and final weight as the first week represents 20% of a chick's total production cycle. Thus proper feed is essential during this time especially the types of fats used as an energy source. During day five and six post-*hatch*, the chick's pancreas experience a lag phase of enzyme activity (lipase, trypsin and chymotrypsin) which decreases the ability of chicks to utilize certain nutrients like fats effectively (Lilburn & Loeffler, 2015). It is well known that diets that contain lipids that are high in SFA, for example tallow, can cause digestive and absorption problems in young chicks (Azman,Konar & Seven 2004). Tallow contains the highest concentration of long-chain SFA (68%) and consists mostly of SFA C16:0 (Palmitic acid) and C18:0 (Stearic acid) (Zhang *et al.*, 2011). Long-chain SFAs that are on the sn-1 and sn-3 positions (example is palmitate) are thought to be less absorbed when compared to the acids on the sn-2 positions. Acids on the sn-2 chain are more hydrophilic and are more readily hydrolysed by lipase than acids on the sn-1 and sn-3 position (Smink *et al.*, 2004). It is therefore important that the type of lipids use as an energy source should be compatible with the physiological ability of the chick at the different stages of growth.

2.6 Different types of feed additives

In-feed antibiotics, also known as antimicrobial growth promoters, have been used world-wide for many years as a mechanism for improving the health, well-being, growth rate, feed conversion efficiency in production animals (Huyghebaert *et al.*, 2011) and to control necrotic enteritis (NE) in poultry. Due to the development of antibiotic resistance, the use of antibiotics as growth promoters were banned in the European Union (EU) (Oso *et al.*, 2013) which led to the research into alternative feed additives such probiotics, prebiotics, medium chain fatty acids, organic acids, emulsifiers, phytogenic feed additives and Anticoccidials. These alternative additives were used as a means to help improve animal performances such as increased feed intake, modulation of gut microbial population and defence against pathogenic bacteria (van der Aar *et al.*, 2016).

Probiotics can be described as live microbial feed provided to animals to improve its intestinal microbial balance (Fuller, 1989). Probiotics are administered in small amounts to the animal (Tellez *et al.*, 2012) to enhance beneficial microorganism and in doing so these microorganism can produce enzymes that could enhance the animal's digestive ability and provide a barrier against invading

microorganisms that are pathogenic (McDonald *et al.*, 2011). In poultry, the use of probiotics have shown to improve the immune system of broilers (Smialek *et al.*, 2018), the reduction of unwanted pathogenic bacteria in the gut (Tellez *et al.*, 2012) and improvement in the body weight gain (BWG) of broilers (O'Dea *et al.*, 2006). Examples of probiotics include *Lactobacillus*, *Enterococcus*, *Bacillus* and *Saccharomyces* (Gaggìa *et al.*, 2010). Prebiotics consist of a group of nutritional modifiers such as oligosaccharides (grape seed meal, legumes, soybean meal), that function by modifying beneficial microbes within the gut to create a healthier environment in the intestine (McDonald *et al.*, 2011). The use of prebiotics have shown to improve average daily gain (ADG) of broilers (Wang *et al.*, 2018), and improved young broiler immunity during the first week *post*-hatch (Huff *et al.*, 2015). Medium chain fatty acids (MCFAs) are low molecular weight tryglycerides that have six to eight carbon atoms within their structure (Saeidi *et al.*, 2016). The most commonly used MCFAs include caproic acid (C6:0, hexanoic acid) and caprylic acid (C8:0, octanoic acid) (van der Aar *et al.*, 2017) and when used as a feed additive have shown to improve lipase activity and therefore aided in lipid digestion, reduced the susceptibility of broilers to pathogenic bacteria (van Gerwe *et al.*, 2010) by potentially altering the intestinal environment so that less favourable pathogenic bacteria are able to proliferate (Solis De Los Santos *et al.*, 2008) and acted as effective antimicrobial agents against Campylobacter without any negative effect on broiler growth performance (Molatová *et al.*, 2011).

Organic acids are regarded as weak and short-chain acids that act as a bacteriostatic agent (for many species) by reducing the pH of the diet to inhibit the growth of unwanted bacteria in the feed (Costa *et al.*, 2013). Examples of organic acids used include propionic and formic acid (Boroojeni *et al.*, 2014), fumaric and citric acid (McDonald *et al.*, 2011), lactic acid (Neal-McKinney *et al.*, 2012) and citric acid (Shah *et al.*, 2018). The use of organic acids have shown to improve lipid utilization by reduced abdominal fatness in broilers and in the improvement in broiler health (Ramigani *et al.*, 2006). Phytogenic feed additives (PFA) are also known as botanical products and consists, for example, of herbs, spices and essential oils and due to the differences of botanical origin, processing and composition (Hafeez *et al.*, 2016) they can have more than one type of mode of action. These can include acting as antimicrobial and ant-viral agents, improving feed intake and flavour, the stimulation and secretion of gastric juices, increasing gastric motility and having anti-inflammatory and anti-oxidative activity (Kirkpinar *et al.*, 2011). Examples of PFA include thymol, eugenol and carvacrol (Agostini *et al.*, 2012), oregano and garlic essential oil (Kirkpinar *et al.*, 2011) and rosemary and sage oil (Lopez-Bote *et al.*, 1998). The use of PFA in broiler production has shown to improve growth performance (Pirgozliev *et al.*, 2019), improvement in nutrient utilization (Hafeez *et al.*, 2016), reduction in pathogenic bacteria count (Kirkpinar *et al.*, 2011) and improvement in feed efficiency (Agostini *et al.*, 2012). Emulsifiers by definition are surfactive substance that acts on the surface between two media that are considered immiscible (e.g. water and oil) (Tan *et al.*, 2016). The dietary lipids consumed by animals are insoluble in the aqueous environment of their gastrointestinal tract and require the action of bile and lipase for lipid digestion (Siyal *et al.*, 2017a). During the first week *post*-hatch, young chicks have limited bile and lipase secretion and therefore are not able to break down lipids effectively (Upadhaya *et al.*, 2017). Therefore, emulsifiers are used to act as a catalyst to

break down dietary fats and to enhance the action of lipase during lipid hydrolysis (Upadhaya *et al.*, 2018). Another advantage in the use of emulsifiers is that it can be used as a tool to administer fat-soluble vitamins to animals via an aqueous medium (Namur et al., 1988).Different types of emulsifiers have been used in animal feed and include 1,3 diacylglycerol (Upadhaya *et al.*, 2017), polyethylene glycol riconoleate (Tan *et al.*, 2016), lysophospholipids (Zampiga *et al.*, 2016), Liprex (Aguilar *et al.*, 2013) and Lysoforte booster (Melegy *et al.*, 2010). In broiler production, the use of emulsifiers have shown to improve feed efficiency (Khonyoung *et al.*, 2015), improved nutrient utilisation (Allahyari-Bake & Jahanian, 2017) and have shown to improved body weight gain (Zhang *et al.*, 2011). Anticoccidials are drugs (Daugschies *et al.*, 1998) that are administered to poultry as a means to prevent coccidiosis (Karlsson & Reid, 2019). Coccidiosis is caused by *Eimeria* (parasite) that invades the cells of the intestine that can lead to diarrhoea and mortality in birds (Mansoori *et al.*, 2009).Common anticoccial drugs used include nicarbazin, narasin, halofuginone, salinomycin and monensin (Daugschies *et al.*, 1998). In the study of Daugschies *et al.* (1998), the use of the different anticoccidiols showed varying results; the use of monesin and nicarbazin reduced the production of coccidia and lesions in the cloaca of broilers, whereas nicarbazin was able to completely eradicate *Eimeria* from the infected flock. The use of salinomycin and narasin has also shown to prevent necrotic enteritis (presented as lesion in the intestines of poultry) caused by *Clostridium perfringens in* broilers (Lanckriet *et al.*, 2010). Overall, there are different types of feed additives that are being used as alternative mechanisms to substitute antibiotics with each conferring various advantages to the performance of an animal. In this study, focus will be placed on the use of different emulsifiers as feed additives and their effect on broiler performance.

2.6.1 Examples of natural emulsifiers

Bile salts are natural endogenous emulsifiers responsible for the emulsification of fats into triglycerides and phospholipids in the duodenum tract of broilers (Doreau & Chilliard, 1997) and also play an important role in improving the action of lipase for the hydrolysis of lipids into triglycerides and monoglycerides needed for micelle production (Upadhaya *et al.*, 2018). When supplemented in the diet of broilers, bile salts improved the average daily gain and improved the final weight of broilers when compared to the control (without bile salts) (Lai *et al.*, 2018). Another study focused on the use of pig bile as a natural emulsifier in the diet of broilers receiving a high fat diet (Lammasak *et al.*, 2018). An improvement in lipase activity and in the total bile acid concentrations were found in broilers receiving pig bile as a supplement. Furthermore, improvements in fat and protein digestibility were also found. Another common natural emulsifier used to improve broiler performance is lecithin. Lecithins are naturally occurring emulsifiers consisting of phosphatidylcholine with different fatty acids that can include oleic, stearic and palmitic acids and are commercially produced from plant oils seeds (sunflower and soybean oil) or can be of animal origin (milk, brain tissue and egg yolk) (Oke *et al.*, 2010). When provided in the diet of broilers, the supplementation of lecithin improved lipid digestion (Woodgate & Van der Veen, 2014), improved feed intake, improved daily gain and growth (Siyal *et al.*, 2017b) and have also shown to regulate fat metabolism in broilers (Huang *et al.*, 2008). Proteins such as caseins and whey proteins have been used for many decades as emulsifiers in the emulsion of food products such as milk, ice cream and various dairy products (Kralova & Sjöblom, 2009).

Casein is another naturally occurring polymeric emulsifier (Dickinson, 1993) and is commonly found in bovine milk (constitutes about 80% of milk protein) (Kralova & Sjöblom, 2009). When supplemented as a feed additive in broiler production, it has shown to improve weight gain, FCR and improved pancreatic lipase activity (Neto *et al.*, 2011). Globin is another naturally occurring protein emulsifier used as a feed additive in broiler production. Dietary globin is known under the name of Actipro ® Globin (Veos, 8750 Zwevezele-Belgium). It's a protein-based emulsifier that contains active hydrophilic protein and is made from porcine blood during red cell fractionation and has similar properties to that of soy lecithin (Dabbou *et al.*, 2019). When used as an emulsifier in the diet of broilers, improvements in fat digestibility, protein metabolism, FCR and in the net energy production were found.

2.6.2 Examples of synthetic emulsifiers

Sodium stearoyl-2-lactylate (SSL) is a synthetic emulsifier and is a sodium salt consisting of a long-chain carbolylic acid with two esters linkages with a very high hydrophilic-lipophilic balance and is a good fat-in water emulsifier (Cho *et al.*, 2012). Sodium stearoyl-2-lactylate is formed by the esterification of stearic acid with lactic acid which is then neutralized to form sodium salt (Gheisar *et al.*, 2015). When added to the diet of broilers, improvement in feed conversion ratio and in the digestibility of energy and nitrogen where found (Gheisar *et al.*, 2015). Furthermore, when added to a diet of low energy improvements in the average daily gain were also found to the same level as that of diets with a high energy level (Cho *et al.*, 2012). Diacylglycerol (DAG) is another synthetic emulsifier consisting of 70% medium chain fatty acids and 30% fatty acids (Upadhaya *et al.*, 2017). The amphiphilic ability of DAG is responsible for its ability to take-up free fatty acids that are not efficiently broken down by bile salts (Dierick & Decuypere, 2004). When added to the diet of broilers, improvement in FCR, ADG and in dry matter digestibility were found (Upadhaya *et al.*, 2017). Lysophosphatidylcholine (LPC), also known as lysolecithins, is derived by enzymatic conversion of lecithin (Jansen *et al.*, 2015). Lysolecithins are considered better emulsifier agents when compared to bile due to its emulsification capacity (Zhang *et al.*, 2011). There are various types of lysolecitihins depending on the lecithin source and can include soybean and rapeseed lecithin (Jansen *et al.*, 2015), from sunflower seeds and from animal sources of which can include milk, eggs and brain tissue (Oke *et al.*, 2010). When used in the diet of broilers, lysolecithins were able to improve the digestibility and the energy of broiler feeds containing saturated fat sources (Jansen). Furthermore, improvements in the FCR and in fat absorption were found in broilers when supplemented in their diets irrespective of fat type (Khonyoung *et al.*, 2015). Other commercial emulsifiers used in broiler production include Lipidol ® Ultra (Zampiga *et al.*, 2016), AVI-MUL TOP (AMT) (Bontempo *et al.*, 2018), Lysoforte Booster ® (Melegy *et al.*, 2010) and Volamel Extra ® (Tan *et al.*, 2016).

2.7 Emulsifier and its function in lipid digestion in young broilers

An emulsifier is a small molecule surfactant that possess both a hydrophilic and a hydrophobic part and is termed amphiphilic (Dickinson, 1993). In a colloid system that comprises of two individual phases that do not mix (for example oil and water), the contact region between the two phases is termed the interface (Norn, 2014). It is at this interfacial region at which emulsifiers are able to exert

their amphiphilic properties by dissolving with its hydrophilic part in water and its hydrophobic part in the oil droplet (Zhao *et al.*, 2015). In other words, the hydrophilic and hydrophobic moieties of emulsifiers enable its absorption to the interfacial region for the stabilisation of the colloid system (Norn, 2014). The degree of lipid or water solubility of the emulsifier is dependent on its hydrophilic-lipophilic balance (HLB) and can range from 0 to 20 (Siyal *et al.*, 2017a). A low HLB is an emulsifier with a higher hydrophobic character whereas a high HLB is an emulsifier that has a higher lipophilic character; therefore emulsifiers with a range of two till six is suited for water in oil systems (continuous phase is oil) and emulsifiers with a HLB of eight and higher is suited for oil in water systems (continuous phase is water) (Norn, 2014). The intestinal tracts of birds are an aqueous environment (Kaczmarek *et al.*, 2015) due to birds consuming 1.5-2 times more water than fats (Siyal *et al.*, 2017a). Therefore, emulsifiers with an HLB range of eight and higher will be suited for the intestinal environment of birds. Example of emulsifiers used in broiler production include sodium stearoyl-2-lactylate which has a HLB of ten (Upadhaya *et al.*, 2018), polyethylene glycol riconoleate has a HLB of greater than 18 (Tan *et al.*, 2016) and Tween 20 has a HLB of 16.7 (Upadhaya *et al.*, 2018).

The breakdown of dietary fats into lipid droplets in the watery environment of the intestines in birds increases the interfacial tension between water and oil (McClements & Jafari, 2017). The use of an emulsifier can reduce this tension by attaching itself with its charged head to one or more of the fatty acids on the lipid droplet (Siyal *et al.*, 2017a). This encourages the dispersion of oil in water to create a stabilised emulsion and the prevention of oil droplet coalescence that can lead to the breakdown of the emulsion (Figure 2.5) (McClements & Jafari, 2017). Furthermore, not all dietary lipids are readily broken down especially in young broilers which negatively affects lipid absorption (Gheisar *et al.*, 2015). Young broilers *post*-hatch lack the needed digestive enzymes to breakdown dietary lipid (Lilburn & Loeffler, 2015). In order for fats to be absorbed, they must first be broken down during lipolysis and incorporated into micelles (Krogdahl, 1985a). Therefore, the formation of micelles greatly influences how effective fats are absorbed and therefore controls how well the products of lipolysis are absorbed across the small intestine (Dierick & Decuypere, 2004). Emulsifiers are able to assist in lipid digestion and absorption in young broilers by acting as a catalyst in breaking lipid down and increasing the surface area of fats enhancing the action of lipase which helps to hydrolyze triglyceride molecules and favours the formation of micelles (Upadhaya *et al.*, 2018). Emulsifiers also help in the prevention of lipid coalescence by stabilising the distribution of lipid droplets in the emulsion to enhance lipid absorption (Zhao *et al.*, 2015) by creating a charge on the surface of the droplet thereby decreasing the physical contact between the droplets and possible coalescence (Siyal *et al.*, 2017b).

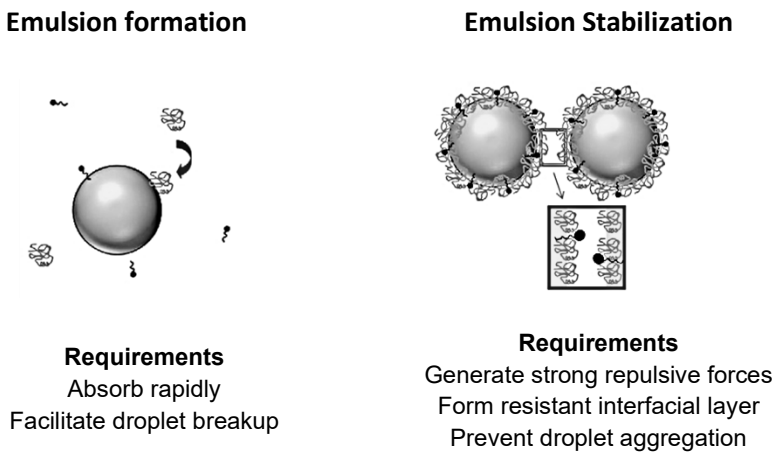

Figure 2.3 Emulsifiers action in an emulsion (McClements & Jafari, 2017)

2.7.1 The effect of emulsifiers on broiler production

Various studies have assessed the effects of different emulsifiers (natural and synthetic) on various production and digestibility performance indicators in young broilers. The supplementation of emulsifiers in broiler diets showed positive effects on live weight (Roy *et al.*, 2010; Siyal *et al.*, 2017b), body weight gain (BWG) (Neto *et al.*, 2011; Zhang *et al.*, 2011; Kaczmarek *et al.*, 2015; Boontiam *et al.*, 2017),feed conversion efficiency (FCE) and in the feed conversion ratio (FCR) of broilers (Roy *et al.*, 2010; Zhang *et al.*, 2011; Khonyoung *et al.*, 2015). The use of an emulsifier also showed improvement in the overall growth performance of broilers throughout the production period (Boontiam *et al.*, 2017; Upadhaya *et al.*, 2017). Emulsifiers can also be utilised as a tool in improving low metabolizable (ME) diets so that the performances of broilers could be similar to those broilers given high ME diets (Kaczmarek *et al.*, 2015; Tan *et al.*, 2016). Positive effects of emulsifiers in energy efficiency has been observed in ME and crude protein (CP) improvement due to enhanced digestion and absorption of dietary lipids and other nutrients (Roy *et al.*, 2010); the apparent metabilizable energy (AME) of starter and grower diets improved when an emulsifier was used (Zhang *et al.*, 2011). Improvement in nitrogen digestibility has also been indicated with the use of an emulsifier (Gheisar *et al.*, 2015). Blends of two different types of emulsifier has shown to improve feed efficiency, body weight gain (BWG) and improvements in dry matter (DM) and lipid digestibility (Upadhaya *et al.*, 2018). The use of emulsifiers had no negative effect on meat quality (Aguilar *et al.*, 2013; Zampiga *et al.*, 2016; Upadhaya *et al.*, 2017) but may have an effect on meat colour (Bontempo *et al.*, 2018). Improvement in lipase production and secretion has also been shown with the use of an emulsifier (Zhang et al., 2011) thereby encouraging lipid digestion.

2.8 Conclusion

A chick's performance throughout its production cycle is impacted by how well it can utilize the nutrients within the feed. During the first few days *post*-hatch, a chick's digestive system is still underdeveloped and early feeding is essential for gastrointestinal tract (GIT) development and the development of microbial gut flora which plays an important role in the digestion and health of broilers. However, it can take up to three weeks for bile secretion to reach adult levels. The physiological inability of the GIT to utilize dietary lipids effectively can be aided in the use of dietary emulsifiers. Emulsifiers can provide the needed emulsification of dietary fats that cannot be provided by the underdeveloped GIT and enables the chick to utilize the energy obtained more effectively for growth and maintenance. The use of emulsifiers also encourages the chick's lipase enzymatic activity in lipid digestion thereby making lipid absorption more efficient.

Therefore, the current study aims to evaluate the effects of three different emulsifiers' together with a sustainable oil source on the production parameters, carcass characteristics, digestibility parameters and gizzard health on broilers under the same experimental conditions (ingredients and nutrient composition, emulsifier levels and environmental temperature) to eliminate the effects external factors may have on the results. These findings would help aid in the better understanding on feed additives and the use of an alternative lipid source on the overall production ability of broilers from *post*-hatch until the age of slaughter. Through this better feeding strategies can be implemented that are more specific to the age of the bird throughout the production period (starter, grower and finisher) and insight on how to possibly maximize each growth phase effectively.

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Chapter 3

An evaluation on the effects of three different dietary emulsifiers and the use of black soldier fly larvae oil on broiler production parameters

Abstract

The objective of the study was to investigate the effects of three emulsifiers and black soldier fly larvae oil on the production parameters of young broilers. Five treatments were used in the trial and consisted of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton, Lysoforte (LYS) at 250g/ton. A thirty two day experiment was performed in which a total of three hundred broilers were used in the trial and randomly assigned to one of the five treatments. Each treatment was replicated 10 times with a total of 60 birds. The weights and feed intake were measured on a weekly basis from day zero till day thirty two from which the feed conversion ratio (FCR) and the European Production efficiency factor (EPEF) were calculated. No significant differences in the growth rate, average daily gain (ADG), FCR, weekly feed intake, PEF, liveability and in the EPEF were found between the different treatments. The results of the study suggest that LO could be used to substitute SF in broiler production without having to negatively affect the production potential of broilers. Furthermore, none of the emulsifiers used in the study showed to have an effect on the production potential of broilers.

***keywords:** ADG, EPEF, FCR, feed intake, growth rate

3.1 Introduction

Over the last few decades, marked improvements has been seen within the broiler industry across various areas which include nutrition, genetics, management and health (Vieira & Angel, 2012). Maintaining or increasing the high growth rate and feed efficiency are the two main targets in broiler production (Sugiharto, 2016). The reason for these improvements are driven by demand and supply (Narrod *et al.*, 2008). With many technological changes seen in the broiler production practices, the industry has been able to reduce their retail prices in comparison to other animal protein sources (Hoffmann, 2019). However, even with advances in poultry practices, feed still remains the major cost component for production (Yegani & Korver, 2008) and can account for about 60-70% of the costs (Ding *et al.*, 2016). In the diets of broilers energy is the major cost component (Siyal *et al.*, 2017a). In order to increase the energy density of broiler diets, fats and oils have been used by nutritionists as an energy-yielding ingredient to improve productivity (Huang *et al.*, 2007). The energy of the diet provided by lipids has been shown to impact feed intake and therefore feed conversion ratio (FCR) by broilers, as broilers adjust their feed intake according to their energy needs (Leeson *et al.*, 1996). The use of emulsifiers can allow for the use of low energy feeds while still maintaining the same performances as that from high energy feeds (Siyal *et al.*, 2017a) thereby improving lipid utilization (Abbas *et al.*, 2016). An improvement in lipid utilization can help reduce the cost of feed thereby allowing for more economical production. The supplementation of emulsifiers in the diet of broilers has yielded positive results; improvements have been seen in body weight and feed efficiency (Kaczmarek *et al.*, 2015; Khonyoung *et al.*, 2015; Upadhaya *et al.*, 2018), in nutrient utilization of the

diet (Melegy *et al.*, 2010; Zhang *et al.*, 2011), an increase in lipase secretion needed for lipid digestion and improvements in energy utilization (apparent metabolizable energy) (Neto *et al.*, 2011). Most of the studies done on emulsifier used commercial lipids of which included sunflower oil (Zampiga *et al.*, 2016), soybean oil (Boontiam *et al.*, 2017) tallow (Upadhaya *et al.*, 2018) and palm oil (Aguilar *et al.*, 2013). There is a lack of research on the study of emulsifiers with the use of black soldier fly larvae oil and its effects on broiler production parameters.

Therefore, the objective of the study was to evaluate the effect of black soldier fly oil without and emulsifier and black soldier fly oil with an emulsifier (EEP, LES and LYS) to sunflower oil as the control on the production parameters of broilers. The parameters include the feed intake, weight gain, average daily gain (ADG), feed conversion ratio (FCR), Protein efficiency factor (PER) and European Production Efficiency Factor (EPEF).

3.2 Materials and methods

3.2.1 Treatment and experimental diets

The experiment consisted of five treatments which included the use of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton and Lysoforte (LYS) at 250g/ton. The BSF larvae oil used was produced by Agriprotein Technologies (Pty) Ltd under the product name of Magoil. The oil was extracted from heat treated larvae of black soldier fly (*Hermentia illucens*). Each treatment was replicated six times (six cages) with ten birds per cage. A completely randomised design was used. All five treatments were formulated according to the Cobb 500 nutrient specifications guide (Cobb Vantress, 2012b). The vitamin and mineral premix were provided at the levels set by the National Research Council (1994). All three emulsifiers were added during the mixing of the diet at the Mariendahl experimental farm of Stellenbosch University. All the diets were provided as mash diets to the bird according to the diet phase. The three diet phases included the starter, grower and finisher (Table 3.1). The main difference between the different treatments is the inclusion of the different emulsifiers at the recommended manufacturers guide (Table 3.2).

Table 3.1 Ingredients and calculated nutrient composition of broiler starter, grower and finisher diets used in the trial

		Starter	Grower	Finisher
Ingredients	**units**			
Maize	%	42.10	48.292	42.10
Soya bean (46%)	%	42.11	36.867	42.11
L-lysine (HCl)	%	0.52	0.231	0.520
DL-methionine	%	0.46	0.369	0.460
L-threonine	%	0.16	0.116	0.160
Vit+min Premix	kg/ton	3.00	3.000	3.000
Limestone	%	1.60	1.331	1.298
Salt	%	4.6	0.109	0.115
Monocalcium phosphate	%	2.13	1.878	1.734
Sodium bicarbonate	%	0.44	0.358	0.355
Black soldier fly oil	%	10	10	10
Sunflower oil	%	10	10	10
Excential energy plus	g/ton	250	250	250
Lesitol	L/ton	0.2	0.2	0.2
Lysoforte Booster	g/ton	250	250	250
Calculated nutrient composition (DM basisi)				
Dry matter	%	89.844	89.544	89.300
AMEn chick	MJ/kg	13.689	13.914	14.123
Crude fat	%	12.131	12.288	12.436
Crude fibre	%	3.132	3.006	2.843
Crude protein	%	23.914	21.690	19.393
Ash	%	4.596	4.095	3.778
Calcium	%	1.050	0.900	0.850
Lysine	%	1.727	1.368	1.200
Methionine	%	0.787	0.681	0.608
Cysteine	%	0.383	0.363	0.338
Methionine + Cysteine	%	1.171	1.043	0.945
Threonine	%	1.057	0.936	0.835
Tryptophan	%	0.286	0.258	0.224
Arginine	%	1.621	1.465	1.378
Isoleucine	%	1.082	0.981	0.862
Histidine	%	0.627	0.579	0.521
Phenylalanine	%	1.086	0.995	0.886
Tyrosine	%	0.939	0.856	0.759
Phenylalanine + Tyrosine	%	2.025	1.851	1.645
Valine	%	1.183	1.087	0.974
Leucine	%	1.971	1.854	1.712
Total Phosphorous	%	0.923	0.844	0.787
Available phosphorous	%	0.500	0.450	0.420
Sodium	%	0.160	0.160	0.160
Chloride	%	0.160	0.160	0.160
Potassium	%	0.989	0.906	0.808

LO: Black Soldier fly larvae oil at 10%; EEP: Excential Energy Plus at 250 g/ton; LES: Lesitol at 0.2L/ton; LYS: Lysoforte at 250 g/ton; SF: sunflower oil at 10%, Dry matter (DM), Apparent metabolizable energy nitrogen corrected (AMEn)

Table 3. 2 Description of the five different treatments used in the starter, grower and finisher phase

Treatment[1]	Inclusion	Description
LO	10%	Main lipidsource
SF	10%	Control
EEP	250g/ton	Standard inclusion
LES	0.2L/ton	Standard inclusion
LYS	250g/ton	Standard inclusion

[1]Black soldier fly larvae oil (LO), Sunflower oil (SF), Excential energy plus (EEP), Lesitol (LES), Lysoforte (LYS)

3.2.2 Birds and housing

A total of 300 1-day old broilers (Cobb 500) were collected at a local hatchery and transported to Mariendahl experimental farm of Stellenbosch University (Stellenbosch, Western Cape , South Africa) were the study took place. The day old chicks, upon arrival, were weighed in groups of ten and placed randomly to one of the five treatment groups. A total of 30 pens were used in the trial. The cages were 1.86m^2 in size and elevated 1.7m off the floor. Each cage was equipped with two nipple drinkers and a feeder but bell drinkers were supplied from the start of the trial until birds could independently drink from the nipple drinkers. The temperature and the lighting of the house were controlled in accordance to the Cobb 500 management guide (Cobb-Vantress, 2012a). The protocol of the study was approved by the Animal Ethics Committee of Stellenbosch University, reference number: ACU-2017-0433-307.

The starter diet was provided at 900g/bird over a period of 14 days with the grower at 1200g/bird and finisher at 1500g/bird until slaughter at day 32. The individual feed intake was calculated as an average with the correction of any mortality. Mortalities were recorded twice daily and weights of dead birds recorded. The live weight of each pen was also recorded on a weekly basis till day 32 of slaughter from which the individual live weight was calculated as the average weight of the pen after correction of any mortalities.

3.2.3 Management and handling of birds

The broilers were cared and managed based on the Cobb 500 management guide (Cobb-Vantress, 2012a) throughout the trial. The first week of the trial the birds were monitored every two hours in which a routine check-up was done to ensure the birds showed normal behaviour patterns of which include being active, eating and drinking and visually assessing their comfort towards the temperature in the house. The total numbers of birds per cage were counted hourly to ensure the correct numbers of birds were in the cage and any mortality was recorded. From the second week the birds were monitored every four hours except during the dark period.

3.2.4 Production data collection

On day 0, 1-day old chick's weights were recorded in groups of ten. On day 7, 14, 21, 28 and 32 the weights of each pen was recorded from which the individual weights was expressed as the average weight of the pen (corrected for mortalities). The weekly feed intake was also recorded on day 7, 14, 21 and 28. Throughout the trial mortalities and the weights were recorded. On day 32, one bird per

cage corresponding to the average weight of the cage was selected and slaughtered. Once slaughtered the carcass quality, organ and intestinal measurements were measured.

The following equations were calculated from the data collected: Average live weight (equation 1), weekly feed intake, cumulative feed intake, feed conversion ratio (FCR)(equation 1), average daily gain (ADG) protein efficiency factor (PER) (equation 2) and the European production efficiency factor (EPEF) (equation 3).

Equation 3.1 Feed conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Cumulative feed intake (g)}}{\text{Average live weight per chick (g)}}$$

Equation 3.2 Protein efficiency factor (PER)

$$\text{PER} = \frac{\text{Weight gain (g)}}{(\text{Weekly feed intake (g) x protein \% of diet})/100}$$

Equation 3.3 European production efficiency factor (EPEF)

$$\text{EPEF} = \frac{\text{Liveability \% × Live weight (g)}}{\text{Age (days) × Feed conversion ratio}} \times \frac{100}{1}$$

3.2.5 Statistical analysis

The statistical analysis was done using statistical analysis software (Statistica, version 13). One-way ANOVA's were conducted to compare treatments in separate analyses per time point. Normal probability plots were investigated to check for deviations from normality, and were in all cases found to be acceptable. Levene's test was done to test for homogeneity of variance assumptions, and all found to be acceptable. For post hoc testing, Fisher Least Significant Difference (LSD) testing was done.

3.3 Results and discussion

3.3.1 Live weight and average daily gain

The effects of LO, EEP, LES and LYS on broiler production parameters are shown in Table 3.4. No significant differences in the growth for the starter, grower and finisher period were found between the different treatments. None of the emulsifiers used in study had improved the growth of broilers when compared to LO and SF and is in agreement with the studies of Zampiga *et al.* (2016), Upadhaya *et al.* (2017), Roy *et al.* (2010) and Dabbou *et al.* (2019). The improvement in lipid digestion by an emulsifier is influenced by the lipid source used in the diet. Jansen *et al.*, (2015) reported significant improvement when the lipid source was of animal origin (saturated fatty acids) with only slight improvements when the lipid source was of plant origin (unsaturated fatty acids). Saturated fatty acids found in animals fats are digested less efficiently than unsaturated fatty acids found in plant fats (Upadhaya *et al.*, 2017). Black soldier fly larvae contains high amounts of linoleic acid and is similar to many plant oils such as soybean oil and sunflower oil (Schiavone *et al.*, 2018). From the current

study, the inability of the emulsifiers to improve the production parameters could be a result of broilers' ability to already digest LO efficiently. This argument is further supported in that LO performed on the same level as SF demonstrating the ability of broilers to utilize LO on the same level as SF. Furthermore, Butcher & Nilipour (2018a) reported that for profitable broiler production, the weight of broilers at day 35 should be at 2kg. All the treatments in the current study had final weight values > 2kg which demonstrates the ability of broilers to utilize LO as an energy source on the same level as SF wihout the aid of an emulsifier. Therefore, LO could be used to substitute SF in broiler production without negatively impacting broiler growth. The average daily gain is the amount of weight a broiler gains per day over a specific period of time. No significant differences (P=0.38) in broiler growth were observed from day 0 till day 32 between the different treatments. On day 7, 14, 21, 28 and 32, no significant differences were also found between the different dietary treatments (Table 3.1). The lack of differences found between LO and SF shows the ability of LO to provide efficient energy for growth throughout the production period. None of the emulsifiers used were able to improve the ADG when compared to LO and SF and agrees with the studies of Bontempo *et al.*, (2018), Khonyoung *et al.* (2015), Neto *et al.* (2011) and Roy *et al.* (2012). Therefore, LO could be used to substitute SF in broiler production without having a negative effect on broiler growth and without the supplementation of an emulsifier.

3.3.2 Feed intake, feed conversion ratio and protein efficiency factor

The effects of LO, EEP, LES and LYS on the weekly and cumulative feed intake is shown in Table 3.4. In the current study, no significant difference in the feed intake and in the cumulative feed intake per week was observed from day zero till day thirty two between the different dietary treatments. The intake of feed by broilers is influenced by the energy concentration of the feed and a constant energy intake is maintained as long as other nutrients are present at the correct levels (Classen, 2016). Birk *et al.* (2016) reported that an increase in lipid inclusion level of 6% and 8% resulted in a decrease in feed intake compared to the control containing lipid at 1.33%. Therefore, the provison of lipid at 10% could have already provided the required energy needed by broilers and that the supplementation of an emulsifer could have less effect on feed intake. In the current study, the lack of differences found between LO and SF could demonstrate the ability of broilers to digest LO as efficiently as SF without an emulsifer therefore contributing to the lack of differences found between LO with LES, EEP and LYS. The supplementation of dietary emulsifiers have shown to improve nutrient utilisation by broilers (Boontiam *et al.*, 2017) rather than on feed intake therefore, as long as the nutrient composition in the diet is provided at the correct levels then the energy of the diet remains the driving force influencing the amount of feed consumed by broilers (Classen, 2016). Therefore, the use of LO as a lipid source could be used to substitute SF in broiler production without having a negative effect on broiler feed intake. The feed conversion ratio accesses how much feed is provided and how much live weight is produced from the feed (Equation 2). The cost of feed accounts for 60-70% of the production cost in poultry production (Ding *et al.*, 2016). The more meat produced on less feed the more desirable, therefore, the lower the FCR the more profitable the production will be. No significant differences were observed in the FCR between the different dietary treatments. This is expected due to the lack of differences (P > 0.05) found in the feed intake and weight gain between the different treatments. The

supplementation of LO as a lipid source performed (P > 0.05) on the same level SF indicating that the use of LO could produce the same economic outcome in terms of the conversion of feed to meat in broiler production. This is further seen in that no significant differences were found between LO with EEP, LES and LYS. The supplementation of emulsifiers to the diet of broilers is postulated to improve the FCR of broilers (Roy *et al.*, 2010); however, in the current study LO was still able to perform on the same level as SF without an emulsifier. The inability of the emulsifiers to improve the FCR in the current study is in agreement with those of (Aguilar *et al.*, 2013), (Melegy *et al.*, 2010) and Zhang *et al.* (2011) who all found no significant differences in the FCR with the supplementation of an emulsifier. Butcher & Nilipour (2018b) reported that for optimum broiler production, a FCR of 1.75 is required. All the treatments in the current study had FCR that fell below this value, therefore, the use of LO without an emulsifier is able to obtain FCR values for optimum broiler production.

Over the years, more consumers are becoming health conscious and are therefore becoming sensitive to the type of food that they consume (Imran *et al.*, 2014). Nutritionally, poultry meat is low in fat, low in sodium and cholesterol levels and is high in unsaturated fatty acids making poultry meat an ideal protein source for consumers that are health conscious (Petracci & Cavani, 2012). Protein supplementation in broiler diets is essential for the provision of essential amino acids for muscle growth (Beski *et al.*, 2015). However, low energy diets can cause amino acid deficiency by directing more amino acids to be used as an energy source rather than to be used for muscle growth negatively impacting muscle production (Classen, 2016). The protein efficiency ratio (PER) is used to determine the amount of weight gain (muscle growth) based on the amount of protein consumed over a set period of time (equation 3.2). In the current study, no significant differences in the PER were observed between the different dietary treatments. The use of LO in the diet of broilers with and without the use of an emulsifier still performed on the same level as SF. In the study of Kijparkorn (2007), the provision of a low energy diet with an emulsifier improved the protein efficiency of broilers when compared to broilers receiving only the low energy diet (no emulsifier). In the current study, the lack of differences (P > 0.05) found between EEP, LES and LYS with LO shows that efficient energy was already provided by LO without the aid of an emulsifier and that the energy consumed was potentially used more for muscle growth than for energy. According to Wilding *et al*. (1968), for optimum protein efficiency in broiler production the ratio should be 3:1. All the dietary treatments in the current study had PER that exceeded the standard of 3:1 for optimum protein efficiency. Therefore, LO could be used to substitute SF in broiler production without negatively effecting broiler protein efficiency without the aid of an emulsifier.

Table 3.3 Average (± standard deviation) of weekly live weights (g), weekly feed intrake (g), cumulative feed intake (g), average daily gain (g), feed conversion ratio and the European production efficiency factor of broilers grown from day 0 till 32

	Treatment					
Production days	**LO**	**SF**	**EEP**	**LES**	**LYS**	**P-value**
Week 0 (Day 0)						
Average live weight	44.25 ± 1.53	44.75 ± 0.90	45.69 ± 2.45	45.80 ± 3.02	43.95 ± 0.90	0.37
Week 1 (Day 7)						
Average live weight	176.63 ± 6.45	174.23 ± 10.46	173.72 ± 6.68	178.61 ± 11.99	176.90 ± 5.69	0.85
Weekly feed intake	142.00 ± 19.66	130.05 ± 11.30	137.67 ± 8.85	135.73 ± 7.41	132.85 ± 4.73	0.46
Cumulative feed intake	142.00 ± 19.66	130.05 ± 11.30	137.67 ± 8.85	135.73 ± 7.41	132.85 ± 4.73	0.46
Week 2 (Day 14)						
Average live weight	502.50 ± 24.44	495.83 ± 22.45	471.7 ± 17.46	481.7 ± 13.08	487.5 ± 6.92	0.19
Weekly feed intake	483.78 ± 34.41	480.13 ± 32.19	475.05 ± 24.56	475.45 ± 10.08	457.45 ± 9.34	0.42
Cumulative feed intake	625.74 ± 20.54	610.18 ± 39.93	612.72 ± 28.86	611.29 ± 13.50	590.30 ± 5.83	0.09
Week 3 (Day 21)						
Average live weight	1132.5 ± 20.44	1092.9 ± 24.67	1080.0 ± 17.46	1079.4 ± 28.43	1083.3 ± 18.01	0.42
Weekly feed intake	645.45 ± 15.43	652.18 ± 38.69	672.18 ± 25.72	667.25 ± 37.25	710.18 ± 54.28	0.13
Cumulative feed intake	1129.23 ± 23.21	1132.31 ± 50.37	1147.23 ± 43.57	1142.81 ± 43.57	1167.63 ± 54.64	0.72
Week 4 (Day 28)						
Average live weight	1775.0 ± 53.74	1790.7 ± 58.04	1832.7 ± 19.418	1749.4 ± 46.09	1807.5 ± 35.42	0.74
Weekly feed intake	914.35 ± 50.89	858.80 ± 42.14	918.52 ± 54.44	839.54 ± 86.58	854.17 ± 68.07	0.18
Cumulative feed intake	1559.80 ± 48.07	1510.97 ± 47.07	1590.70 ± 43.05	1506.79 ± 43.05	1564.35 ± 104.94	0.20
Week 5 (Day 32)						
Average live weight	2237.2 ± 47.28	2194.4 ± 30.18	2219.5 ± 21.37	2121.8 ± 53.15	2205.8 ± 38.65	0.32
Weekly feed intake	1373.017 ± 50.91	1339.4 ± 60.64	1372.933 ± 38.07	1312.68 ± 27.27	1351.800 ± 60.64	0.13
Cumulative feed intake	2287.37 ± 99.51	2198.20 ± 91.36	2291.45 ± 82.68	2152.22 ± 108.58	2205.97 ± 143.50	0.10
FCR	1.59 ± 0.07	1.58 ± 0.0.05	1.61 ± 0.0.05	1.62 ± 0.06	1.59 ± 0.03	0.56
ADG	62.63 ± 3.89	61.37 ± 2.70	60.34 ± 3.89	57.55 ± 4.08	61.77 ± 2.70	0.11
PER	3.24 ± 0.13	3.27 ± 0.11	3.20 ± 0.10	3.19 ± 0.11	3.25 ± 0.06	0.59
EPEF	432.30 ± 21.59	421.31 ± 41.78	423.30 ± 21.59	403.06 ± 34.36	433.77 ± 19.07	0.34

[(a,b)] Means with different superscripts within the same row differ significantly (P<0.05); [1] LO: Black soldier fly larvae oil control; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte, FCR (feed conversion ratio), ADG (Average daily gain), PER (protein efficiency ratio), EPEF (European production efficiency factor)

3.3.4 European production efficiency factor and liveability

The standard inclusion of lipid in the diet of broilers is at 2 to 8% (McDonald *et al.*, 2011). Lipid inclusion levels that > 8% could result in digestive disturbances and diarrhoea impacting the production ability of birds (Ayed *et al.*, 2015). According to Butcher & Nilipour (2018), the first week of the production period a mortality rate of 0.80% and a mortality rate of < 4% for the entire production period is required for efficient broiler production. The mortality rate of the first week was at 0.67% and the mortality rate for the entire production period was 1.67% which is below that of 0.80% and 4%. In the current study, the liveability percentage for LO, SF, EEP, LES and LYS was 98.33%, 96.67%, 98.33%, 98.33% and 100% respectively. The statistical analysis for the liveability between the different dietary treatments showed no variance in the data points. The lack of differences indicates that the use of LO at 10% and the supplementation of EEP, LES and LYS had no negative effect on broiler liveability. This is further supported in chapter 5 of this study that showed the use of LO and SF at 10% and the use of EEP, LES and LYS at the standard and at the double the standard inclusion level had no negative effect on broiler gizzard health. Gizzard erosion in broilers can be caused by certain components within the diet and if not treated can cause up to 20% mortality rate in the first week *post* hatch (Fossum *et al.*, 2008). Studies done on the use of emulsifiers have also shown that the supplementation of emulsifiers in broiler diets do not negatively affect broiler liveability (Melegy *et al.*, 2010; Aguilar *et al.*, 2013; Zampiga *et al.*, 2016) and in fact has shown to improve broiler liveability (Cho *et al.*, 2012). Therefore, LO could be used to substitute SF in broiler production without negatively affecting broiler liveability and that the use of emulsifiers EEP, LES and EEP in the diet of broilers had no negative effect on broiler liveability.

Factors that can impact the production potential of broilers include health, nutritional requirements, environmental conditions and management (Butcher & Nilipour, 2018b). The European production efficiency factor (EPEF) is used to determine the production efficiency of broilers by taking into account the liveability, live weight, age and FCR. The EPEF is a good tool in assessing LO compared to SF and an emulsifier's ability to improve the production efficiency of broilers. Butcher & Nilipour (2018) reported that to obtain an efficient production of a 40 day production period the follow needs to be achieved: EPEF value ≥ 360 units, ADG value ≥ 65g, FCR value ≤ 1.75 and a slaughter weight of 2.5kg. In the current study, no significant differences in EPEF were observed between the different treatments. The EPEF for all treatments were above the stated standard of 360 units. The lack of differences found between LO and SF shows that LO is able to support for efficient broiler production as that of SF even without the use of an emulsifier. The lack of differences found between LO with EEP, LES and LYS shows that none of the emulsifiers were able to improve the EPEF therefore further demonstrating the ability of LO to support for efficient broiler production. The values obtained in the current study is in agreement with the EPEF obtained by Cockcroft (2018) with the use of LO. Therefore, LO could be used to substitute SF in broiler production without negatively effecting broiler production efficiently.

3.4 Conclusion

The results from the study showed that the use of LO as a sustainable energy source when compared to SF had no negative effect on broiler production parameters which include growth rate, feed intake, cumulative feed intake, ADG, FCR, PEF, EPEF. Furthermore, none of the emulsifiers used in the current study was able to neither improve nor negatively affect the various broiler production parameters (growth rate, feed intake, cumulative feed intake, ADG, FCR, PEF, EPEF). Therefore, LO could be used to substitute SF in broiler production.

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Chapter 4

An evaluation on the effects of three different dietary emulsifiers and the use of black soldier fly larvae oil on broiler carcass characteristics

Abstract

The objective of the study was to investigate the effects of three emulsifiers and black soldier fly larvae oil on the production parameters of young broilers. Five treatments were used in the trial and consisted of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton, Lysoforte (LYS) at 250g/ton. A thirty two day experiment was performed in which a total of three hundred broilers were used in the trial and randomly assigned to one of the five treatments. Each treatment was replicated ten times with a total of 60 birds. No significant differences in the live weight, warm carcass weight, cold carcass weight, dressing percentage, carcass portions, breast component yields and in the pH (initial and ultimate) of the breast and thigh meat were found between the different dietary treatments. The breast colour also did not differ between the treatments; however, differences in thigh meat yellowness (b*) and chroma were found between the different dietary treatments. The results of the study could suggest that the supplementation of the different emulsifiers and the use of LO had no effect on the various carcass characteristics and pH but may have had an effect on thigh b*.

***keywords**: dressing percentage, meat colour, meat pH

4.1 Introduction

Over the years broilers have been selected in breeding programmes so that they reach slaughter weight in a shorter period of time. The demand for poultry meat has added pressure on the poultry industry to produce birds that grow faster, have improved feed efficiency, larger breast muscle production and less abdominal fatness (Petracci & Cavani, 2012). Furthermore, this demand in poultry meat is attributed to various factors of which include an increase in household income, increases in pork and beef prices, increases in the preference of poultry meat (low lipid and sodium animal protein) and the amount of poultry products available on the market such as whole chickens, portioned, deboned and ready-to-eat processed products. The consumers perception of poultry meat is highly influenced by appearance, texture, juiciness and flavour and use meat colour as an indication of wholesomeness (Mancini & Hunt, 2005) and will often reject meat products deviating from what is considered as normal appearance (Qiao *et al.*, 2001). The colour of meat is assessed based on three colours and include lightness (L*), redness (a*) and yellowness (b*) (Chan *et al.*, 2011). Pale, soft and exudative (PSE) and dark, firm and dry (DFD) are both unwanted conditions that greatly impacts the quality of broiler meat (Droval *et al.*, 2012; Ristic & Damme, 2013). PSE meats tend to have a pH of 5.77, normal meat has a pH of 5.89 and DFD meat tend to have a pH of 6.04 (Petracci *et al.*, 2004). The colour of meat can therefore be used as a tool to differentiate between light (PSE), normal and dark (DFD) broiler meat and the effects it may have on the functional and physical properties of meat (Qiao *et al.*, 2001).

Emulsifiers are classified as a feed additive that is added to the diet of broilers to act as a catalyst to enhance lipid digestion and absorption (Upadhaya *et al.*, 2018) and has been shown to improve broiler production which include growth (Upadhaya *et al.*, 2018), dressing percentage (Melegy *et al.*, 2010) and meat production (Boontiam *et al.*, 2017). Most of the studies done on emulsifier used commercial oils which include, sunflower oil (Zampiga *et al.*, 2016), soybean oil (Boontiam *et al.*, 2017) tallow (Upadhaya *et al.*, 2018) and palm oil (Aguilar *et al.*, 2013). There is a lack of research on the study of emulsifiers with the use of black soldier fly larvae oil and the effects on broiler carcass characteristics.

Therefore, the objective of the current study is to evaluate the effects of three different emulsifiers and the use of black soldier fly larvae oil, a sustainable oil source, on the carcass characteristics of young broilers. The comparative measurements include dressing percentage, carcass component yield, meat pH and meat colour.

4.2 Materials and methods

4.2.1 Treatment and experimental diets

The experiment consisted of five treatments which included the use of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton and Lysoforte (LYS) at 250g/ton. The BSF larvae oil used was produced by Agriprotein Technologies (Pty) Ltd under the product name of Magoil. The oil was extracted from heat treated larvae of black soldier fly (*Hermentia illucens*). Each treatment was replicated ten times (per cage) with a total of 60 birds. A completely randomised design was used. All five treatments were formulated according to the Cobb 500 nutrient specifications guide (Cobb Vantress, 2012b). The vitamin and mineral premix were provided at the levels set by the National Research Council (1994). All three emulsifiers were added during the mixing of the diet at the Mariendahl experimental farm of Stellenbosch University. All the diets were provided as mash diets to the bird according to the diet phase. The three diet phases included the starter, grower and finisher (Table 3.1). The main difference between the different treatments is the inclusion of the different emulsifiers at the recommended manufacturers guide.

4.2.2 Carcass characteristics

On day 32, before slaughter, the selected birds live weights were recorded. After which the birds were slaughtered followed by defeathering, removal of head and feet and evisceration. The initial pH (pH_i), using a calibrated portable Crison pH25 meter (Alella, Barccelona), of the right breast and thigh were recorded 15 minutes *post mortem*. The carcasses were then hung in cold storage at 4 $^{\circ}$C for 24 hours. After 24 hours, the ultimate pH (pH_u) of the breast and thigh was measured in the same manner and position as the pH_i. After 24 hours in cold storage the carcasses cold weight were recorded. The cold carcass was then cut up into the commercial portions; thigh, breast, wing and drumstick. The carcass were first cut in half, the thigh and drumstick (as a whole) were separated by cutting above the thigh

towards the acetabulum and behind the public bone, the thigh and drumstick were then removed by cutting perpendicular towards the joint connecting the two cuts and the wings were removed by cutting between the scapula and the coracoid. The different cuts were then weighed (Mettler PC 4400 scale (Mettler- Toldeo, Switzerland) to determine the percentage portion yields as the weight portions to the cold carcass weight.

After the portions were obtained the CIE- Lab colour meter was used to determine the L *, a * and b * of the breast and thigh and were measured in triplicates. The a* and b *values of the breast were used to calculate the hue angle (h_{ab}) (°) and chroma value (C^*). The tissue yields of the breast and thigh were removed as follows: The skin and subcutaneous lipidwas removed and weighed, the meat was weighed and, the bone was removed from the meat weighed. All the component yields were expressed a percentage relative to the portion weight.

Equation 4.1 Dressing percentage (D %)

$$\text{D \%} = \frac{\textbf{Warm carcass weight (g)}}{\textbf{Live weight (g)}} \boldsymbol{x\ 100}$$

Equation 4.2 Portion yield percentage (PY %)

$$\text{PY \%} = \frac{\textbf{Portion weight (g)}}{\textbf{Cold carcass weight (g)}} \textbf{ x 100}$$

Equation 4.3 Breast component yield (BCY %)

$$\text{BCY \%} = \frac{\textbf{Breast component (g)}}{\textbf{Total breast compinent (g)}} \textbf{ x 100}$$

4.2.3 Statistical analysis

The statistical analysis was done using statistical analysis software (Statistica, version 13). For cases where there was one measurement per cage, one-way ANOVA's were conducted to compare treatments. Normal probability plots were investigated to check for deviations from normality, and were in all cases found to be acceptable. Levene's test was done to test for homogeneity of variance assumptions, and all found to be acceptable. For post hoc testing, Fisher Least Significant Difference (LSD) testing was done.

4.3 Results and discussion

4.3.1 Dressing percentage

The dressing percentage is one of many factors that have an impact on the value of the slaughtered animal. The dressing percentage gives an indication of the amount of muscle, bone and fat produced at the end of the production period (day 32) (Equation 4.1). The effects of LO and the different emulsifiers on the dressing percentage are shown in Table 4.1. The supplementation of emulsifier EEP, LES and LYS had no effect (P > 0.05) on the dressing percentage when compared to LO and

SF and is in agreement with Aguilar et al. (2013) and (Roy *et al.*, 2010) who found no differences in the dressing percentage when an emulsifier was used. Emulsifiers are supplemented in the diet of young broilers to help aid in lipid digestion and utilization for improved growth (Boontiam *et al.*, 2017; Upadhaya *et al.*, 2018) but in the current study, the lack of differences found in the live weight, warm carcass weight and in the cold carcass weights between LO and EEP, LES and LYS indicates the ability of young broilers to utilized LO efficiently without the help of an emulsifier. This is further supported by the lack of differences ($P > 0.05$) found between LO and SF and is in agreement with the study of Cockcroft (2018) who found no differences in dressing percentage between black soldier fly larvae oil and sunflower oil.The current findings of the study indicate that the addition of the different emulsifiers to the diet did not perform better than LO and that broilers were able to utilise LO efficiently on the same level as SF which could indicate that LO could be a substitute for SF in broiler production without having a negative effect on the dressing percentage.

4.3.2 Carcass component and breast tissue yield

A main focus of broiler production is to maximise the genetic potential of broilers so that the yield of primarily cuts such as the breast and thigh could be increased. The increased demand for poultry meat has added pressure on the poultry industry to produce broilers with increased growth rate, feed efficiency, breast muscle size and the reduction in abdominal fatness (Petracci & Cavani, 2012). The effects of the five different treatments on the carcass portion yield (g) are shown in Table 4.2. The supplementation of emulsifiers EEP, LES and LYS had no effect ($P> 0.05$) on the portion yields of the breast, thigh, drumstick when compared to LO and SF. Similarly, Aguilar *et al.* (2013), Roy *et al.*, (2010), Neto *et al.* (2011) and Melegy *et al.* (2010) reported that the use of dietary emulsifiers had no effect on the carcass yield of broilers. Even when an emulsifier was supplied at double the concentration level, this led to no effect on the breast and thigh yield (Melegy *et al.*, 2010). Furthermore, the lack of differences ($P > 0.05$) found between LO and SF is consistent with the findings of Cockcroft (2018). Similarly, Schiavone *et al.* (2017a) also reported no differences in carcass portion yields with the use of LO when compared to soybean oil. Therefore, none of the emulsifiers used improved the dressing percentage of broilers. Furthermore, the abilty of LO to perform on the same level as SF indicate that LO could be used to substitute SF in broiler production without having an effect on broiler dressing percentage.

The breast tissue yield is comprised of the muscle, skin with fat and bone. These components are expressed as a percentage of the breast (Equation 4.3) and are given in Table 4.2. The supplementation of emulsifiers EEP, LES and LYS had no effect ($P > 0.05$) on the breast tissue yields when compared to LO and SF and is consistent with the findings of Upadhaya *et al.* (2017), Aguilar *et al.* (2013), Upadhaya *et al.* (2018), Cho *et al.* (2012) and Neto *et al.* (2011). No differences ($P > 0.05$) in the breast components were also found between the LO and SF and are in agreement with the study of Cockcroft (2018). The lack of differences between LO and SF indicates that LO performed on the same level as SF and could possibly be used as substitute for SF in broiler production without having a negative impact on broiler carcass portion yields.

Table 4.1 Average (± standard deviation) of live weight (g), warm carcass weight (g), cold carcass weight (g) and warm dressing percentage (%) from broilers slaughtered at 32 days of age that received the five different dietary treatments

Treatments[1]	Live weight	Warm carcass weight	Cold carcass weight	Dressing %
LO	2249.42 ± 162.77	1544.83 ± 105.03	1500.92 ± 100.52	66.78 ± 2.17
SF	2151.17 ± 116.29	1501.58 ± 112.43	1460.25 ± 106.34	67.89 ± 3.55
EEP	2176.58 ± 75.24	1466.08 ± 75.32	1424.75 ± 70.47	65.50 ± 3.27
LES	2120.42 ± 142.96	1488.08 ± 115.44	1456.92 ± 113.02	68.71 ± 2.61
LYS	2188.58 ± 105.41	1512.42 ± 67.71	1484.58 ± 67.20	67.86 ± 1.66
P-value	0.14	0.37	0.34	0.06

[1]LO: Black soldier fly larvae oil; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte

Table 4.2 Average (± standard deviation) of carcass portion yields from broilers slaughtered at 32 days of age that received the five different dietary treatments

	Treatments					
	LO	**SF**	**EEP**	**LES**	**LYS**	**P-value**
Carcass portion yields						
Breast (g)	463.33 ± 42.02	461.33 ± 48.83	423.42 ± 32.74	455.42 ± 42.85	457.33 ± 46.25	0.17
Thigh (g)	453.92 ± 32.43	426.83 ± 26.15	434.50 ± 28.81	436.00 ± 40.15	451.50 ± 30.43	0.37
Drumstick (g)	207.25 ± 19.31	206.67 ± 15.44	191.67 ± 25.92	208.92 ± 21.84	207.42 ± 17.23	0.36
Wings (g)	280.42 ± 27.44	270.50 ± 31.17	275.08 ± 22.05	272.75 ± 25.48	264.42 ± 25.14	0.76
Breast (%)	30.85 ± 1.49	31.55 ± 1.84	29.73 ± 1.93	31.30 ± 2.00	30.76 ± 2.29	0.21
Thigh (%)	30.25 ± 1.08	29.27 ± 1.24	30.40 ± 1.09	29.93 ± 1.53	30.40 ± 1.26	0.15
Drumstick (%)	13.82 ± 1.09	14.17 ± 0.73	13.47 ± 1.80	14.32 ± 0.69	14.97 ± 0.99	0.46
Wing (%)	18.73 ± 1.88	18.50 ± 1.36	19.30 ± 1.11	18.73 ± 1.24	17.84 ± 1.95	0.33
Breast component yields						
Skin & lipid(%)	5.87 ± 1.17	5.80 ± 0.98	6.04 ± 1.05	6.06 ± 1.65	6.29 ± 1.12	0.92
Bone (%)	12.34 ± 1.07	12.40 ± 1.77	14.42 ± 2.72	13.67 ± 4.17	13.33 ± 1.78	0.25
Meat (%)	78.90 ± 3.35	76.75 ± 4.68	77.24 ± 6.67	78.21 ± 6.08	80.43 ± 4.94	0.57

LO: Black soldier fly larvae oil; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte

Table 4.3 Average (± standard deviation) of colour measurements and pH from broilers slaughtered at 32 days of age that received the five different dietary treatments

Parameters	Treatments[1]					
	LO	SF	EEP	LES	LYS	P-value
Breast colour						
L*	58.43 ± 4.88	60.51 ± 4.47	58.43 ± 4.88	59.13 ± 4.12	58.72 ± 4.47	0.49
a*	5.93 ± 15.80	11.40 ± 21.72	1.37 ± 1.40	1.15 ± 0.90	1.52 ± 2.03	0.50
b*	7.41 ± 2.05	8.67 ± 2.86	8.49 ± 1.90	8.36 ± 2.10	8.09 ± 2.41	0.40
Hue	80.53 ± 9.68	78.37 ± 9.35	81.78 ± 6.49	82.48 ± 5.11	79.88 ± 12.08	0.62
Chroma	4.13 ± 0.69	4.51 ± 0.90	4.39 ± 0.66	4.32 ± 0.62	4.32 ± 0.78	0.52
Thigh colour						
L*	59.61± 4.02	63.21 ± 4.42	58.70 ± 4.07	60.08 ± 5.60	60.05 ± 4.64	0.13
a*	2.76 ± 2.24	3.56 ± 1.48	2.62 ±1.62	3 .26 ± 2.45	2.44 ±1.59	0.11
b*	7.63^{a} ± 2.21	9.77^{c} ± 1.88	7.90^{a} ±1.81	9.33^{bc} ± 2.31	8.53^{ab} ± 2.01	**0.01**
Hue	71.05 ± 12.82	69.73 ± 9.15	71.62 ± 12.32	71.58 ± 12.36	73.88 ± 12.36	0.68
Chroma	4.49^{c} ± 0.78	5.14^{a} ± 0.48	4.56^{c} ± 0.55	4.96^{ab} ± 0.75	4.65^{bc} ± 0.55	**< 0.00**
pH						
pH_i breast	6.13 ± 0.24	6.07 ± 0.27	6.03 ± 0.31	6.17 ± 0.27	6.21 ± 0.24	0.56
pH_u breast	5.80 ± 0.05	5.79 ± 0.14	5.79 ± 0.90	5.80 ± 0.11	5.81 ± 0.07	0.95
pH_i thigh	6.98 ± 0.10	5.87 ± 0.14	5.99 ± 0.09	6.02 ± 0.19	5.93 ± 0.25	0.15
pH_u thigh	6.06 ± 0.14	5.95 ± 0.11	6.02 ± 0.14	6.05 ± 0.17	6.12 ± 0.25	0.22

[1]LO: Black soldier fly larvae oil; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte, initial pH (pH_i), ultimate pH (pH_u

4.3.3 pH and CIE-lab measurements

Consumers use meat colour as a major quality factor for the determination of meat freshness and wholesomeness (Mancini & Hunt, 2005). The colour of meat is strongly influenced by pH (Wideman *et al.*, 2019) and it is widely accepted that there is an inseparable relationship between colour and pH (Mancini & Hunt, 2005). The effects of the five different treatments on the pH and meat colour are shown in Table 4.3. No differences (P >0.05) in the pH_I and pH_u for both the breast and thigh muscle were found with the use of EEP, LES and LYS when compared to LO and SF and is in an agreement with Upadhaya *et al.* (2015) and Upadhaya *et al.* (2018). Lack of differences (P >0.05) in pH_I and pH_u of the breast and thigh muscle were also found between LO and SF and agrees with Cockcroft (2018) and Schiavone *et al.*, (2017). According to Fletcher (1999), the pH of lighter meat is 5.63, pH of normal meat is 5.70 and the pH of darker meat is 5.81. All treatments in the current study had pH values > 5.81 for both the breast and thigh (Table 4.3). Normal meat is associated with L* values of 50 till 56, darker meat is associated with L* < 50; lighter meat is associated with L* > 56 (Petracci et al., 2004). All treatments in the current study showed L* values above 56 for both the breast and thigh indicating lighter meat, which contradicts the expectation of the higher pH values; the latter being associated with DFD meat. No significant differences in the breast meat L*, a*,b*, chroma and hue were found between LO and SF and are in agreement with the study of Cockcroft (2018). No

significant differences were also found in breast meat L*, a*, b*, chroma and hue values were found between EEP, LES and LYS when compared to LO and SF. Similarly, Upadhaya *et al.* (2017),Aguilar *et al.* (2013) and Zampiga *et al.* (2016) found no differences in breast meat colour when an emulsifier was used. Interestingly, significant differences were found in thigh b* and chroma values between the different treatments. Plant oils such as SF are high in unsaturated fatty acids and are more readily digestible than saturated fatty acids found in animal fats (Upadhaya *et al.*, 2017). The high b*(9.77) and chroma (5.14) value of SF could be due to the increase in xanthophyll digestibility with an increase in SF digestibility, therefore contributing to the thigh meat colour that is more yellow in colour (more saturated). It was reported by Bontempo *et al.* (2018), the use of an emulsifier containing lecithin resulted in breast meat with higher b* value than the control due to the ability of emulsifiers to increase lipid-soluble pigments of which include xanthophyll found in maize when provided in the diet. The plant oil component found in LES may have contributed to the increase in xanthophyll digestibility in the similar way as that of SF contributing to an increase in b* and chroma value. However, there is a lack of research on the effects of emulsifiers on carotenoid absorption.

4.4 Conclusion

The results from this study showed that the supplementation of LO in the diet of broilers has no effect on broiler carcass dressing percentage, carcass portion yields, breast component yields and on the pH and colour and hue of the breast and thigh meat and could replace SF in broiler production. The supplementation of emulsifiers in the diet of broilers showed to have no negative effect on broiler carcass dressing percentage, carcass portion yields, breast component yields and on the pH on the breast and thigh meat but may have an effect on thigh meat colour.

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Chapter 5

An evaluation on the effects of three different dietary emulsifiers and the use of black soldier fly larvae oil on broiler organ and intestinal parameters

Abstract

The objective of the study was to investigate the effects of three different emulsifiers and the use of black soldier fly larvae oil on the organ and intestinal measurements of young broilers. The gizzard health of the broilers was also evaluated using gizzard erosion scores. Five treatments were used in the trial and consisted of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton, Lysoforte (LYS) at 250g/ton. A thirty two day experiment was performed in which a total of three hundred broilers were used in the trial and randomly assigned to one of the five treatments. Each treatment was replicated ten times with a total of 60 birds. No significant differences were found for the following (including the weights and the organ to body percentage): gizzard, liver, heart and spleen; the pH of the duodenum, ileum and cecum; the red (a*) and yellow (b*) colour ordinates of the liver, and the gizzard scores between the different treatments. Significant differences were found in the weight of the bursa of Fabricius, pH of the gizzard and jejunum and in the lightness (L*) colour ordinate of the liver. The results of the study indicate that the supplementation of the different emulsifiers and the use of LO showed minimal effects on the various intestinal, organ and their measurements but LO and emuslifer type could have an effect on liver colour.

***keywords**: organ weight, organ to body percentage, intestinal pH, gizzard erosion

5.1 Introduction

Poultry health plays a crucial role in the success of production. Importantly, during a range of days *post*-hatch feeding is needed for the development of various organs responsible for maintaining health. For instance, early provision of feed is essential for the development of intestinal and gut-associated lymphoid tissue (Yegani & Korver, 2008). Organs that play an important role in the immune response of poultry are known as lymphoid organs and include the spleen, bursa of Fabricius and thymus (Khoso *et al.*, 2017). The bursa of Fabricius is an organ that is unique to birds and is responsible for the increased production and differentiation of B lymphoid precursors (Fellah *et al.*, 2008). Furthermore, during the early stages of a chicks life the bursa ducts are responsible for the transportation of antigens from the environment into the bursal lumen and then into the lymphoid follicles to induce an immune response thereby initiating antibody production (Yegani & Korver, 2008). Various factors can contribute to an increase in bursal size. For instance, an infected bursa is characterised by being large in size and weight due to oedema and hyperaemia (Berg, 2010). Infectious bursal disease virus (IBDV) is a disease that targets the bursa of Fabricius and affects its development of B lymphocytes and is more prevalent in birds of three and six weeks of age when the bursa is at its full development (Norman & Cheville, 1967). Gumboro is another disease that affects the bursa of Fabricius, spleen and thymus and causes lymphoid necrosis (Norman & Cheville, 1967).

The spleen in birds plays an important role in the filtering of blood from damaged cells and antigens, the production of lymphocytes as well as the maturation and storage of these cells and cellular immune responses (Smith & Hunt, 2003). The cause of splenomegaly has been more frequently associated with parasitic infections (John, 1994), therefore spleen size has been used as a means to determine the immune strength of poultry (Smith & Hunt, 2003). The liver is another organ that plays an important role in immunity through the detoxification of unwanted substances that can result in severe sickness (Andretta *et al.*, 1934). Furthermore, the liver is considered the major site of amino acid (essential and non-essential) metabolism due to the enzymes found to occur at this site (Lobley, 2003). However, the presence of mycotoxins in the diet of poultry can pose as a threat to liver metabolism (van der Aar *et al.*, 2017).

The gizzard, proventriculus, intestines, caeca and the cloaca all form part of the GIT in poultry. The assessment of these different organs is essential when different feed and feed additives are used. For instance, gizzard erosion is a disease that can effect birds from the first week post-hatch up to five weeks of age and without treatment can cause up to 20% mortality rate (Fossum *et al.*, 2008). With necropsy assessment birds with gizzard erosions contained a thicker and softer koilin layer (a solid layer of carbohydrate protein lining the gizzard) that is lighter in colour than in healthy gizzards (Gjevre *et al.*, 2013). There are a number of factors that can provoke gizzard erosion in broilers. These factors include stress (Džaja *et al.*, 1996), mycotoxins (Contreras & Zaviezo, 2007), high levels of copper sulphate (Keirs *et al.*, 1991), histamine and histamine agonists such as gizzerosine (associated with the overheated fish meal) (Džaja *et al.*, 1996; Kaldhusdal *et al.*, 2012) and adenovirus infection (Lim *et al.*, 2012).

Producers have been utilising additives as a long-term means to improve health throughout the entire production period as opposed to short-term veterinary drugs (Wallace *et al.*, 2010). The utilisation of dietary emulsifiers in broiler production have resulted in improved lipid digestibility (Upadhaya *et al.*, 2018), enhanced broiler immune response to pathogens (Boontiam, Jung, & Kim (2010), improved organ weights (Cho *et al.*, 2012) and possible prevention in gizzard erosion (Keirs *et al.*, 1991). There is a lack of research on the utilization of emulsifiers with black soldier fly larvae oil on organ and intestinal parameters and the overall effect on broiler health.

Therefore, the objective of the current study was to evaluate the effects of three different emulsifiers and the use of black soldier fly larvae oil on the organ and intestinal parameters of young broilers. The comparative measurements include the weight and portions of the heart, liver, gizzard, spleen and the bursa of Fabricius; the pH values of the proventriculus, duodenum, jejunum, ileum and the cecum and on the liver colour measurements (L *, a * and b *). Gizzard health was also be evaluated by the use of gizzard scores.

5.2 Materials and methods

The experiment consisted of five treatments which included the use of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton and Lysoforte (LYS) at 250g/ton. The BSF larvae oil used was produced by Agriprotein Technologies (Pty) Ltd under the product name of Magoil. The oil was extracted from heat treated larvae of black soldier fly (*Hermentia illucens*). Each treatment was replicated ten times (per cage) with a total of 60 birds. A completely randomised design was used. All five treatments were formulated according to the Cobb 500 nutrient specifications guide (Cobb Vantress, 2012b). The vitamin and mineral premix were provided at the levels set by the National Research Council (1994). All three emulsifiers were added during the mixing of the diet at the Mariendahl experimental farm of Stellenbosch University. All the diets were provided as mash diets to the bird according to the diet phase. The three diet phases included the starter, grower and finisher (Table 3.1). The main difference between the different treatments is the inclusion of the different emulsifiers at the recommended manufacturers guide. One bird per cage with the average live weight of the cage was selected and slaughtered. Once slaughtered the organ and intestinal parameters were measured.

A secondary trial (gizzard erosion trial) was performed to evaluate the effects of the different emulsifiers provided at the standard and at double the standard manufacturer's guide (Table 5.1). The objective of study was to confirm that the use of Excential Energy Plus, Lesitol and Lysoforte at the standard inclusion level and at double the standard inclusion level as well as the use of black soldier fly larvae oil do not contain any toxins or irritants that could negatively impact the gizzard health of broilers. Gizzard erosion scores from one till four were used as the comparative measure (Table 5.2). Each treatment was replicated 20 times for the entire experiment thus yielding a total of 160 birds for the experiment. A completely randomised design was used. Treatment diets were formulated according to the Cobb 500 nutrient specifications table (Cobb Vantress, 2012b). All the diets were mixed at the Mariendahl Experimental Farm in Stellenbosch. The BSF larvae oil used was produced by Agriprotein Technologies (Pty) Ltd. under the product name of Magoil. The oil was extracted from heat treated black soldier fly (*Hermentia illucens*). The formulated diets were then provided *ad-libitum* from the start until the end of the trial. The birds used for the trial were collected at a local hatchery and delivered to the Mariendahl Experimental farm (33°51' 0 S; 18° 49'60 E) of Stellenbosch University in the Western Cape, of South Africa. Post-arrival, the chicks were randomly assigned to one of the eight cages. Each cage was provided a commercial starter diet for seven days after which were provided at random one of the eight diets until slaughter at day 18. All the birds were euthanized by cervical dislocation after which the gizzards were removed, cut open longitudinally, the pH measured after which were rinsed and scored according to Table 5.2. The pH was measured using a calibrated (standard buffers pH 4.0 and 7.0 at 25°C) portable Crison pH25 meter (Alella, Barcelona).

5.2.1 Organ weights, intestinal pH and liver colour

On the day of slaughter the heart, liver, gizzard, spleen and bursa of Fabricius were removed and weighed used a Mettler PC 4400 laboratory scale (Mettler- Toledo, Switzerland). The weights of the organs were used to calculate the organ weights relative to the live weight of the bird. Once the gizzard was removed, the pH of the gizzard was measured using a calibrated (standard buffers pH 4.0 and 7.0 at 25˚C) portable Crison pH25 meter (Alella, Barcelona). After the removal of the organs, the duodenum, jejunum and ileum were removed for analysis. The duodenum was cut on the gizzard side, jejunum was cut from the middle section between the duodenum and the ileum and the ileum was cut 5mm from Meckel's diverticulum to the ileocecal junction. After removal, the pH measurements of the duodenum, jejunum, ileum, proventriculus and cecum were taken 15min *post-mortem*. CIE- Lab colour meter was used to determine the L *, a * and b * coordinates of the liver. The a* and b *values of the liver were used to calculate the hue angle (h_{ab}) (°) and chroma value (C^*).

Table 5.1 Description of treatments used from day 7 till day 18 for the Gizzard erosion trial

Treatment	Inclusion	Description
LO	10%	Main lipidsource
SF	10%	Control
EEP1	250g/ton	Standard inclusion
EEP2	500g/ton	Double standard inclusion
LES1	0.2L/ton	Standard inclusion
LES2	0.4L/ton	Double standard inclusion
LYS1	250g/ton	Standard inclusion
LYS2	500g/ton	Double standard inclusion

Table 5.2 Gizzard erosion scoring description (Johnson & Pinedo, 1971)

Score	Description
0	No erosion
1	Light erosion (roughness of epithelia)
2	Modest erosion (roughness and gaps)
3	Severe erosion (gaps and ulcers on stomach wall showing slight haemorrhage)
4	Extreme erosion (roughness, gaps and haemorrhage ulcers on stomach wall and separation of epithelia from stomach wall)

5.2.2 Statistical analysis

The statistical analysis was done using statistical analysis software (Statistica, version 13). One-way ANOVA's were conducted to compare treatments in separate analyses per time point. Normal probability plots were investigated to check for deviations from normality, and were in all cases found to be acceptable. Levene's test was done to test for homogeneity of variance assumptions, and all found to be acceptable. For post hoc testing, Fisher Least Significant Difference (LSD) testing was done.

5.3 Results and discussion

5.3.1 Organ weight and organ to body percentage

The effects of the five different treatments on the weights and portions (organ to body weight percentage) of the gizzard, heart, liver, spleen and bursa of Fabricius are shown in Table 5.3 and 5.4. The supplementation of EEP, LES and LYS had no effect ($P > 0.05$) on weights and portions (organ to body percentage) of the gizzard, liver, heart and spleen when compared to LO and SF and are in agreement with the studies of Luc *et al.* (2010), Neto *et al.* (2011), Roy *et al.* (2010) and Zhao *et al.* (2017). No significant differences were also found in the weights and portions of the measured parameters between LO and SF and agrees with the study of Cockcroft (2018) who found no differences in the measured parameters between LO and SF. In the studies of Schiavone *et al.* (2018) and Wang & Shelomi, (2017), LO could replace 100% of soybean oil without having a negative effect on the measured parameters. The findings of the current study indicates that the use of EEP, LES and LYS had no impact on weight and portions of the measured parameters and performed on the same level of LO and SF. This could indicate that broilers were able to utilize LO efficiently without the use of an emulsifier as LO performed on the same level as SF. Therefore, LO could be used to substitute SF in broiler production without the use of an emulsifier and without having a negative impact on broiler production.

The spleen and the bursa of Fabricius (BF) are known as the lymphoid organs and play an important role in the immune response of poultry (Khoso *et al.*, 2017). It was reported by Cho *et al.* (2012) that a reduction in relative spleen weight in broilers supplemented with an emulsifier could lead to an immunosuppressive effect and thus health problems. In the current study, no differences ($P > 0.05$) were found in the weight and in the portion of the spleen between EEP, LES and LYS with LO and SF indicating that none on the emulsifiers had an immunosuppressive effect on broilers. No significant differences in spleen weight and proportion were also found between LO and SF indicating that the use of LO could be use to substitute SF without having an immunosuppressive effect on broilers and is in agreement with the study of Cockcroft (2018).However, significant differences in BF weight were found between the different treatments. Significant differences in BF weight were found between LO and SF between LO and LES and between SF with LYS. These finding was unexpected due to the lack of differences ($P > 0.05$) in the BF portion (organ to body weight percentage) of broilers and in the BF to spleen ratio (0.94) between the different treatments. An infected bursa is characterised as being oversized due to oedema and hyperaemia (Berg, 2010). Diseases such as the Infectious bursal disease virus (IBDV) and Gumboro can affect the development of B lymphocytes and cause lymphoid necrosis (Norman & Cheville, 1967). However, in disease free birds the increase in the weight of the BF can be correlated with an increase in immune cell production (Teo & Tan, 2007). In the current study the mortality rate of 1,67% fell below the accepted standard mortality rate of 2%. This could indicate that other factors other than infection or diseases may have contributed to the differences in BF weight found between the different treatments. The significantly heavier BF weight (4.7g) of treatment LO could be associated with an improvement in the bird's immune system (increase in immune cell production).It was reported by Nourmohammadi *et al.* (2011), an increase in a lymphoid

organ size can be an indication of an imporvment in the immune status of broilers. Additionally, it was found that an increase in soybean oil in the diet of broilers improved the immune status of broilers and was linked to the increased presence of polyunsaturated fatty acids in the diet (Nayebpor *et al.*, 2007). Furthermore, it is known that both n-6 and n-3 polyunsaturated fatty acids (PUFA) are precursors for eicosanoids such as prostaglandins, tramboxans, leukotrines and lipoxins that are important bioactive hormones of the immunoregulatory response (Nayebpor *et al.*, 2007). Black soldier fly larvae contains high amounts of linoleic acid and is similar to many plant oils such as soybean oil and sunflower oil (Schiavone *et al.*, 2018). In the study of Nayebpor *et al.* (2007), there was an increase in the amount of antibodies with an increase in dietary soybean oil levels and due to the similarities between LO and soybean oil it can be speculated that LO could have enhanced antibody production and therefore increased BF weight when compared to SF. Allahyari-Bake & Jahanian (2017) reported an increase in BF weight with the supplementation of lysolecithin (dietary emulsiflier) when compared to broilers receiving unsupplemented emulsifier diet. Furthermore, Cho *et al.* (2012) also reported an increase in the BF weight when an emulsifer was provided in the diet. Therefore, the supplementation of lysolecithin also increased IBD antibody levels in broilers. It can therefore be suggested that LYS could have improved antibody production in broilers contributing to an increase in BF weight when compared to SF. On the other hand, the differences in BF weight between LO and LES could be as a result of emulsifier and fat type interaction. It was shown that the interaction of lysolecithin with soybean oil resulted in a lower spleen weight; whereas broilers receiving palm fat powder (fat sources) showed improved spleen weight. It can therefore be suggested that the interaction of LO and LES might have had an effect on BF weight therefore contributing to the lower BF weight of LES (3.64g) amoungst the treatments (Table 5.3). Nevertheless, according to Cazaban *et al.* (2015) normal BF weights for broilers at 35 days of age range between 1.68 and 4.0g. Treatments EEP, SF and LES fell within this range with the exception of LO and LYS which could be due to their effect on improved antibody production and therefore on BF weight.

Table 5.3 Average (± standard deviation) of weight (g) of the heart, liver, gizzard, spleen and the bursa of Fabricius of 32 day old broilers fed the five different dietary treatments

Treatments[1]	Heart (g)	Liver (g)	Gizzard (g)	Spleen (g)	Bursa of Fabricius (g)
LO	8.85 ± 0.52	50.58 ± 3.92	27.62 ± 0.90	2.40 ± 0.34	4.68^{a} ± 0.60
SF	9.53 ± 0.47	46.59 ± 2.49	30.00 ± 2.72	2.00 ± 0.25	3.45^{c} ± 0.77
EEP	9.05 ± 0.54	48.30 ± 4.16	26.40 ± 0.81	2.42 ± 0.35	4.02^{abc} ± 0.75
LES	8.58 ± 0.48	44.29 ± 3.83	27.67 ± 1.76	2.20 ± 0.26	3.64^{bc} ± 0.58
LYS	9.02 ± 0.53	49.08 ± 2.67	25.68 ± 0.98	2.53 ± 0.30	4.42^{ab} ± 0.96
P-value	0.76	0.75	0.40	0.75	0.04

(^{a,b}): Means with different superscripts within the same row differ significantly (P<0.05); [1] LO: Black soldier fly larvae oil control; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte

Table 5.4 Average (± standard deviation) of organ to body weight percentage of the heart, liver, gizzard, spleen, bursa of Fabricius and bursa of Fabricius to spleen ratio of 32 day old broilers fed the five different dietary treatments

Treatments[1]	Heart%	Liver%	Gizzard%	Spleen%	Bursa of Fabricius%	Bursa of Fabricius: Spleen
LO	0.36 ± 0.04	2.03 ± 0.14	1.11± 0.04	0.10 ± 0.01	0.19 ± 0.01	0.53 ± 0.21
SF	0.40 ± 0.02	1.97 ± 0.10	1.27 ± 0.11	0.08 ± 0.01	0.15 ± 0.01	0.64 ± 0.32
EEP	0.39 ± 0.02	2.08 ± 0.17	1.14 ± 0.04	0.10 ± 0.01	0.17 ± 0.01	0.61 ± 0.20
LES	0.38 ± 0.02	1.97 ± 0.16	1.24 ± 0.08	0.10 ± 0.01	0.16 ± 0.01	0.63 ± 0.24
LYS	0.38 ± 0.02	2.05 ± 0.12	1.07 ± 0.04	0.11 ± 0.01	0.19 ± 0.02	0.60 ± 0.19
P-value	0.53	0.97	0.24	0.79	0.17	0.94

(^{a,b}): Means with different superscripts within the same row differ significantly ($P<0.05$); [1] LO: Black soldier fly larvae oil control; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte

Table 5.5 Average (± standard deviation) of intestinal pH readings of 32 day old broilers fed the five different dietary treatments

Treatments[1]	Gizzard	Proventriculus	Duodenum	Jejunum	Ileum	Cecum
LO	3.61^{a} ± 0.38	3.56 ± 0.76	6.00 ± 0.16	5.89^{b} ± 0.019	6.67 ± 0.55	6.75 ± 0.30
SF	3.24^{ab} ± 0.51	3.34 ± 0.83	6.22 ± 0.18	6.09^{ab} ± 0.14	6.87 ± 0.45	6.64 ± 0.33
EEP	3.25^{ab} ± 0.40	3.74 ± 0.81	5.84 ± 0.60	6.00^{b} ± 0.22	6.82 ± 0.90	7.06 ± 0.57
LES	2.81^{b} ± 0.50	3.06 ± 0.89	6.26 ± 0.21	6.25^{a} ± 0.15	6.90 ± 0.50	6.44 ± 0.30
LYS	3.57^{a} ± 0.41	3.05 ± 0.51	6.00 ± 0.12	6.00^{b} ± 0.14	7.09 ± 0.45	6.67 ± 0.23
P-value	**0.03**	0.46	0.12	**0.01**	0.80	0.09

(^{a,b}): Means with different superscripts within the same row differ significantly (P<0.05); [1] LO: Black soldier fly larvae oil control; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte

Table 5.6 Number of frequency per gizzard erosion category recorded per treatment group of 32 day old broilers of the production trial

	Treatments [2]				
Score [1]	**LO**	**SF**	**EEP**	**LES**	**LYS**
0	2	4	5	3	3
1	4	1	1	3	2
2	0	1	0	0	1
3	0	0	0	0	0
4	0	0	0	0	0
P-value			0.58		

[2]LO: Black Soldier fly larvae oil; EEP: Excential Energy Plus; LES: Lesitol; LYS: Lysoforte; 1-Standard inclusion level; 2-Double the standard inclusion level [1] Table 5.2 Gizzard erosion scoring description (Johnson & Pinedo, 1971)

Table 5.7 Number of frequency per gizzard erosion category recorded per treatment group for the gizzard erosion trial

	Treatments[1]							
Score[2]	**LO**	**SF**	**EEP1**	**EEP2**	**LES1**	**LES2**	**LYS1**	**LYS2**
0	7	5	4	6	9	5	7	4
1	10	8	14	10	2	6	7	12
2	1	5	0	1	7	3	1	1
3	2	2	2	1	1	3	3	3
4	0	0	0	2	0	1	1	0
P-value	0.54							

1LO: Black Soldier fly larvae oil; EEP: Excential Energy Plus; LES: Lesitol; LYS: Lysoforte; 1-Standard inclusion level; 2-Double the standard inclusion level; 2 Table 5.2 Gizzard erosion scoring description

Table 5.8 Average (± standard deviation) of liver colour measurement (L*, a*, b*) of 32 day old broilers receiving the five different dietary treatments

Parameters	**Treatments[1]**					
	LO	**SF**	**EEP**	**LES**	**LYS**	**P-value**
L*	34.17^{a} ± 3.32	30.91^{abc} ± 3.50	30.69^{bc} ± 3.32	29.05^{c} ± 2.34	33.46^{ab} ± 3.58	**0.02**
a*	13.26 ± 1.56	14.93 ± 1.90	14.43 ± 3.32	10.72 ± 2.73	12.27 ± 1.35	0.68
b*	11.07 ± 3.15	12.83 ± 3.12	10.58 ± 2.73	10.72 ± 2.73	12.27 ± 1.35	0.59
Hue	68.22 ± 14.41	70.08 ± 9.56	67.19 ± 11.42	64.90 ± 6.96	72.87 ± 7.85	0.62
Chroma	6.95 ± 0.57	7.43 ± 0.62	7.17 ± 0.81	6.98 ± 0.71	7.21 ± 0.36	0.62

(^{a,b}): Means with different superscripts within the same row differ significantly (P<0.05); [1] LO: Black soldier fly larvae oil control; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte

5.3.3 Intestinal pH measurements

The gastrointestinal tract (GIT) of broilers is a vital organ for the consumption, digestion and absorption of nutrients. Broilers have relatively acidic gastric environment but factors such as health, types of nutrients in the diet and microorganism can affect the pH. Furthermore, the pH at the different regions of the GIT is essential as it influences the growth of specific microbial population, for example beneficial microorganisms grow in a pH range of 5.8 to 6.2; whereas pathogens grow in a pH of 7 or higher (Rahmani & Modirsanei, 2018). A change in pH can predispose the gut to pathogenic bacteria and diseases as well as affect nutrient digestion and absorption (Bedford et al., 1996). The effect of the different treatments on the pH readings of the gizzard, proventriculus, duodenum, jejunum, ileum and the cecum are shown in Table 5.5. The normal pH of the gizzard can vary between 1.9 to 4.5 with an average of 3.5 (Svihus, 2014).In the current study, emulsifier LES had the lowest pH gizzard reading (2.81 ± 0.50) and differed significantly from LO and LYS. There is a lack of research on the effect of emulsifiers on the pH of the gizzard and explanations can only be done through speculation. According to Guinotte *et al*, (1995), a low gizzard pH improves pepsin activity, increases mineral absorption and improves nitrogen retention ultimately improving feed digestibility and utilization by broilers. Therefore, it can be speculated that the low gizzard pH brought about by LES may have improved feed utilization by broilers. According to Engberg *et al.* (2002) and Nir *et al.* (1993), a negative relationship exists between the gizzard pH and the pH of the small intestine. Even though no significant differences occur in the duodenum pH between the different treatments, treatment LES had the highest duodenum pH which could further support the speculation that LES may have brought about a lower gizzard pH. The increase in pH from the gizzard to the duodenum is as expected. When the acidic content of the gizzard enters the duodenum the duodenal glands produce an alkaline secretion that buffers and protects the duodenal wall from the hydrochloric acid from the upper digestive tract. The pH range of the duodenum is between 5 and 6 (Gauthier, 2002), in the current study tretaments LO, EEP and LYS fell within this range whereas treatments LES (6.22 ± 0.18) and SF (6.22 ± 0.18) were slightly over; however no signifincat differences in the duodenum pH were found between the different treatments.

The jejunum is the second section of the small intestine that is mainly responsible for the absorption of nutrients (McDonald *et al.*, 2011). The pH of the jejunum ranges between 5.5 and 7.7 with an average of 6.6 (Moran, 1982 as cited by Herpol; & Van Grembergen, 1967)). In the current study, even though significant differences in jejunum pH were found between LES with LO, EEP and LYS the high pH of LES (6.25 ± 0.15) still fell within range and could be attributed to the increase in duodenal secretion to buffer against the acidic contents of the stomach thereby increasing the pH. The pH of the ileum is found to be between 5.7 and 8.2 (average of 7.2) and the pH of the cecum is found between 5.7 and 8.4 (average of 6.9) (Moran, 1982 as cited by Herpol & Van Grembergen, 1967). No significant differences in the ileum and cecum pH were found between the different treatments and alll treatments pH values for the ileum and cecum fell within this normal range.

5.3.4 Liver colour

The liver is an essential organ that plays a vital role in the metabolism of fat, protein, carbohydrates, vitamins and minerals, detoxification and the removal of unwanted waste products (Zaefarian *et al.*, 2019). The main site for lipid metabolism (fatty acid synthesis) in poultry is the liver (Doreau & Chilliard, 1997). Almost all lipid accumulation is in the adipose tissue of broilers that are derived from the liver or diet (Jiang *et al.*, 2014). Trampel *et al.* (2005) reported an association with hepatic lipid concentration and liver lightness (L*). It was reported that full fed broilers showed lighter liver colour than broilers who did not receive feed for 12 hours. Therefore, the more lipids accumulated in the liver the lighter the liver colour. In the current study, all birds were fed an *ad libitum* diet; however, significant differences in liver L* colour ordinate were found between the different treatments (Table 5.8). Significant differences in the liver L* colour ordinate were found between LO with EEP and LES. There is a lack of research on the effects on the use of emulsifiers on liver colour measurements. Therefore, the current finding can only be explained through speculation. In the study of Roy *et al.* (2010), broilers fed the control diet (without an emulsifier) showed higher liver lipid accumulation for the trial period than broilers supplemented with an emulsifier. The use of an emulsifier could have assisted in the deposition of fats more towards adipose tissue and a reduction in lipid deposition in the liver (Roy *et al.*, 2010 as cited in Deersjant-Li & Peisker, 2005)). Huang *et al.* (2008) reported that the use of soybean-lecithin as an emulsifier affected the expression of hepatic genes that are involved in lipid metabolism in the liver. In the current study, it can be speculated that LES and LYS might have had an effect on lipid metabolism by reducing lipid accumulation in the liver and possibly assisted the deposition of fats more towards adipose tissue. The higher L* colour ordinate of LO when compared to EEP and LES could be postulated on the finding of Sanz *et al.* (2000). It was found that the use of sunflower oil in the diet of broilers led to a decrease in abdominal lipid percentage by preventing the activity of fatty acid synthase (FAS) in the liver (Sanz *et al.*, 2000). Fatty acid synthase is one of many main enzymes that are involved in lipogenesis and is stimulated by insulin by animals in the fed state (Hermier, 1997). Therefore, the unsaturated fatty acid component of LO could have increased lipid accumulation in the liver as opposed to in the abdominal tissue. This finding is further supported in the lack of differences ($P > 0.05$) in the L* colour ordinate between LO and SF. Therefore, the use of LO in the diet of broilers could reduce lipid accumulation in the abdominal tissue, whereas the use of an emulsifier could promote the lipid accumulation in the abdominal tissue than in the liver. However, due to the lack of research on the effects of emulsifiers on liver lightness, the understanding of these findings is only through speculation.

5.3.5 Gizzard erosion

The gizzard in poultry is mainly associated with the grinding and mixing of feed (Ravindran *et al.*, 2016). The gizzard is an organ that contains a keratinoid-like lining which is essential in protecting the underlying mucosa from the digestive secretions of the proventriculus (Contreras & Zaviezo, 2007) during gut refluxes (movement of digesta with digestive enzymes from the proventriculus back to the gizzard) (Ravindran *et al.*, 2016). A dysfunctional gizzard as a result of damage to the gizzard lining and can cause poor nutrient degradation and can result in too high levels of undigested nutrients

entering the intestines causing morphological and microbiological changes (Svihus, 2014). Various factors can cause damage to the keratinoid-like lining of the gizzard (erosion) and include stress (Džaja *et al.*, 1996), mycotoxins (Contreras & Zaviezo, 2007), high levels of copper sulphate (Keirs *et al.*, 1991), histamine and histamine agonists such as gizzerosine (associated with the overheated fish meal) (Džaja *et al.*, 1996; Kaldhusdal *et al.*, 2012), adenovirus infection (Lim *et al.*, 2012), high *Clostridium perfringens* count (Taylor *et al.*, 2006) and feed structure (Ross, 1979). No significant differences in the gizzard erosion scores in both the production (primary trial) and gizzard trial were observed between the different treatments (Table 5.6 and 5.7) and that the incidences of severe gizzard erosion were low during both trials. This is indicative that the use of LO at 10% and the supplementation of EEP, LES and LYS at the standard and double the standard manufactures guide contained no unwanted substances that could have provoked gizzard erosion in broilers and therefore could be used in broiler production without negatively effecting gizzard health.

5.4 Conclusion

The use of emulsifiers in the diet of broilers had no effect on the organ weight and organ to body weight percentage of the gizzard, liver, spleen and hearts of broilers. However, the use of LO in the diet had an effect on the weight of the bursa of Fabricius (BF). The heavier weight of the BF associated with treatment LO could be due to an improvement in the birds' immune system as a result of an increase in immune cell production. The significantly lower gizzard pH reading of LES still fell within the normal pH range of this organ. The lack of differences observed in the gizzard erosion scores and the low incidences of severe gizzard erosion between the different treatments is indicative that none of the treatments used in the current study contained any unwanted agents that could provoke gizzard erosion in broilers. The high pH of the jejunum of treatment LES still fell within the normal pH range and the high pH could be more due to an increase in duodenal secretion to buffer against the acidic contents of the stomach. The differences in liver lightness found between the different treatments could be due to the effect of emulsifiers on lipogenesis; however there is not enough research on the effects of emulsifiers on liver colour.

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Chapter 6

The effects of different emulsifiers and the use of black soldier fly on the total tract digestibility of young broilers

Abstract

The objective of the study was to investigate the effects of three different emulsifiers and the use of black soldier fly larvae oil on the total tract digestibility of young broilers. The nutrients evaluated included fibre, fat, protein, ash and moisture. The apparent metabolizable energy (AME) of the diets was also evaluated. Five treatments were used in the trial and consisted of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. The three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton and Lysoforte (LYS) at 250g/ton. Each treatment was replicated three times with a total of twenty four birds per treatment. The birds were first acclimatized to a standard control diet for four days (adaption period) after which were individually weighed and grouped into birds of three per cage and randomly assigned to one of the five treatments. After which the digestibility trial commenced for four days from which faecal samples were collected each day. No significant differences were found in the coefficient of total tract digestibility (CTTD) of crude protein, crude lipid and ash between the different treatments. Differences in the the apparent metabilizable energy (AME) and crude fibre were found between the different treatments. Emulsifiers LES improved the AME and crude fibre (CF), whereas EEP significantly decreased AME and the CF digestibility when compared to LO. The differences seen between EEP with LO may be attributed to the emulsifier and lipid interaction.

***keywords:** AME, ash, crude fibre, crude fat, crude protein, CTTD

6.1 Introduction

The main aim of nutritionist is to satisfy the nutrient requirements of broilers so that production targets can be met of which can include maximizing growth, increasing breast meat yield for profitability and reducing feed conversion ratio to save on production cost (Lemme *et al.*, 2004) . Therefore, diets of broilers should be formulated to ensure that broilers are able to utilize the raw ingredients efficiently to achieve these production targets. During the first two weeks of a broiler's life, their small intestine is still undergoing many morphological and biochemical changes (Yegani & Korver, 2008) leading to the inability to utilize the feed provided efficiently. During day five and six post-*hatch*, the chick's pancreas experience a lag phase of enzyme activity (lipase, trypsin and chymotrypsin) which decreases the ability of chicks to utilize certain fats effectively (Lilburn & Loeffler, 2015). This is a challenge for the broiler industry as the first two weeks post-*hatch* represents 20% of the chicks production cycle (Tancharoenrat *et al.*, 2013). It is therefore essential that the raw ingredients provided to broilers during the different stages of growth can be utilized efficiently to maintain effective production. Broiler diets are formulated with specific nutrients to improve the production ability of broiler so that the overall total energy in the feed should provide the bird with the necessary energy needed for various functions. The total energy can be determined by the amount of heat measured when a known unit of feed is completely oxidized and is termed gross energy (GE) (McDonald *et al.*, 2011). The energy that is ultimately available for the bird to utilize is termed apparent metabolizable energy (AME) and is of great importance (McDonald *et al.*, 2011). The apparent metabolizable energy is the amount of

energy provided in the diet minus the amount of energy that remains within the excreta and is therefore the amount of energy the bird has consumed. To determine how well a nutrient component of the feed has been digested can be done through a digestibility trial. Digestibility of the feed can therefore be defined as the amount of the nutrient, presented as a fraction, which is digested and absorbed by the bird (Lemme *et al.*, 2004). In many poultry studies, a known unit of feed is provided for a set period from which the total excreta is collected and analysed to determine various chemical analysis of which include crude protein, crude fibre, fat, moisture, ash and the AME of the diet (Dierick & Decuypere, 2004; Tancharoenrat *et al.*, 2013). Additives such as emulsifiers can have an effect on how dietary nutrients are digested. Emulsifiers can either enhance digestion and/or absorption or decrease its digestive ability. The use of emulsifiers have been shown to improve protein digestibility (Luc *et al.*, 2010; Boontiam *et al.*, 2017),lipid digestibility and on the AME utilization (Roy *et al.*, 2010). Most of the studies done on emulsifier used commercial lipids of which included sunflower oil (Zampiga *et al.*, 2016), soybean oil (Boontiam *et al.*, 2017) tallow (Upadhaya *et al.*, 2018) and palm oil (Aguilar *et al.*, 2013).

Therefore, objective for the current study is to determine the effects of LO and the three different emulsifiers on the digestive abilities of the feed components through the following analysis and include crude protein (CP), acid hydrolysis (AH), crude fibre (CF) and AME (GE).

6.2 Materials and methods

6.2.1 Treatment and experimental diets

The experiment consisted of five treatments which included the use of sunflower oil (SF) as the control, black soldier fly larvae oil (LO) without an emulsifier and black soldier fly larvae oil with the different emulsifiers. Three different emulsifiers used consisted of Excential energy plus (EEP) at 250g/ton, Lesitol (LES) at 0.2L/ton and Lysoforte (LYS) at 250g/ton. The BSF larvae oil used was produced by Agriprotein Technologies (Pty) Ltd under the product name of Magoil. The oil was extracted from heat treated larvae of black soldier fly (*Hermentia illucens*). Each treatment was replicated three times (per cage) with a total of twenty four birds. A completely randomised design was used. All five treatments were formulated according to the Cobb 500 nutrient specifications guide (Cobb Vantress, 2012b). The vitamin and mineral premix were provided at the levels set by the National Research Council (1994). All three emulsifiers were added during the mixing of the diet at the Mariendahl experimental farm of Stellenbosch University. All the diets were provided as mash diets to the bird according to the diet phase (Table 6.1). The main difference between the different treatments is the inclusion of the different emulsifiers at the recommended manufacturers guide.

Table 6.1 Ingredient and calculated nutrient composition of broiler starter diets used in the trial

		Starter
Ingredients	**units**	
Maize	%	42.10
Soya bean (46%)	%	42.11
L-lysine (HCl)	%	0.52
DL-methionine	%	0.46
L-threonine	%	0.16
Vit+min Premix	kg/ton	3.00
Limestone	%	1.60
Salt	%	4.6
Monocalcium phosphate	%	2.13
Sodium bicarbonate	%	0.44
Black soldier fly oil	%	10
Sunflower oil	%	10
Excential energy plus	g/ton	250
Lesitol	L/ton	0.2
Lysoforte Booster	g/ton	250
Dry matter	%	89.844
AMEn chick	MJ/kg	13.689
Crude fat	%	12.131
Crude fibre	%	3.132
Crude protein	%	23.914
Ash	%	4.596
Calcium	%	1.050
Lysine	%	1.727
Methionine	%	0.787
Cysteine	%	0.383
Methionine + Cysteine	%	1.171
Threonine	%	1.057
Tryptophan	%	0.286
Arginine	%	1.621
Isoleucine	%	1.082
Histidine	%	0.627
Phenylalanine	%	1.086
Tyrosine	%	0.939
Phenylalanine + Tyrosine	%	2.025
Valine	%	1.183
Leucine	%	1.971
Total Phosphorous	%	0.923
Available phosphorous	%	0.500
Sodium	%	0.160
Chloride	%	0.160
Potassium	%	0.989

LO: Black Soldier fly larvae oil at 10%; EEP: Excential Energy Plus at 250 g/ton; LES: Lesitol at 0.2L/ton; LYS: Lysoforte at 250 g/ton; SF: sunflower oil at 10%, Dry matter (DM), Apparent metabolizable energy nitrogen corrected (AMEn)

Table 6.2 Description of the five different dietary treatments used in the starter, grower and finisher phase

Treatment[1]	Inclusion	Description
LO	10%	Main lipid source
SF	10%	Control
EEP	250g/ton	Standard inclusion
LES	0.2L/ton	Standard inclusion
LYS	250g/ton	Standard inclusion

[1]Black soldier fly larvae oil (LO), Sunflower oil (SF), Excential energy plus (EEP), Lesitol (LES),

6.2.2 Birds and housing

A total of 120 day old broilers (Cobb 500) were collected and transported approximately 50km to Mariendahl experimental farm of Stellenbosch University (Stellenbosch, Western Cape, South Africa) were the study took place. Post arrivals at the farm, broilers were first grouped together with 10 birds per cage. A commercial diet was provided for six days. On day six broilers were weighed individually and birds with similar mass were grouped into groups of three birds per cage. After which each cage was randomly assigned to one of the five treatments. Therefore, each treatment was replicated eight times (eight cages). The cages were 1.86m in size and elevated 1.7m off the floor. Each cage was equipped with two nipple drinkers and a feeder but bell drinkers were supplied from the start of the trial until birds could independently drink from the nipple drinkers. The temperature and the lighting of the house were controlled in accordance to the Cobb 500 standard guide (Cobb Vantress, 2012a). The protocol of the study was approved by the Animal Ethics Committee of Stellenbosch University, reference number: ACU-2017-0433-307

6.2.3 Management and handling of birds

The broilers were cared for and managed based on the Cobb 500 management guide (Cobb-Vantress, 2012a) throughout the trial. The first week of the trial the birds were monitored every two hours in which a routine check-up was done to ensure the birds showed normal behaviour patterns of which include being active, eating and drinking and visually assessing their comfortability towards the temperature in the house (chicks cuddling together is an indication of the temperature being too low). The total number of birds per cage was counted during each check-up to ensure the correct number of birds were in the cage and any mortalities were recorded. From the second week the birds were monitored every four hours except during the darkness period.

6.2.4 Digestibility data collection

The digestibility trial commenced from day 14 till day 18. During this time, faecal samples and the feed left over (refusal) were collected each day and cleaned from any visible feathers after which was weighed. The samples were then stored immediately in the freezer until further analysis. Before analysis, the faecal samples for each cage were pooled together after which 500g were weighed and dried in an oven for three days. Once dried, the samples were then milled from which the various analyses were performed.

6.2.5 Analytical methodologies

6.2.5.1 Dry matter determination

The dry matter (DM) content of the faecal and feed samples was determined according to the Association of Official Analytical Chemists International (AOAC) (2002), official method 934.01. The samples were dried at 100 °C for 24 hours.

6.2.5.2 Ash determination

The duplicate samples used in the dry matter determination (3.2.4.1) were retained and used to analyse the ash content of the feed and faeces (AOAC, 2002; official method 942.05). The samples were combusted in a furnace oven at 500°C for 6 hours.

6.2.5.3 Crude lipid determination

The crude lipid content of each treatment feed and faeces sample were determined using the acid hydrolysis lipid extraction method using diethyl ether, petroleum ether, ethanol and hydrochloric acid 38% reagent as described by the AOAC (2002), official method 920.39.

6.2.5.4 Crude protein determination

The crude protein content of each treatment feed and faecal samples was determined by measuring the total nitrogen content using a LECO FP528 machine, according to the Dumas combustion method 992.15 described by AOAC (2002). The nitrogen content was directly measured and used to calculate the crude protein content using a factor of 6.25

6.2.5.5 Crude fibre determination

The crude fibre in the feed and faeces samples was analysed according to the official method 962.09 (AOAC, 2002) on a Fibertec/Dosifiber extrusion apparatus. The samples were dried in a 100° C oven for 48 hour and then combusted at 500°C for 6 hours.

6.2.5.6 Gross energy

The gross energy of the feed and faecal samples was determined using the CP 500 isothermal bomb calorimeter. The CP 500 isothermal bomb calorimeter apparatus was calibrated before commencing of analysis. At the start of the analysis two benzoic acid tablets were analysed separately, the values obtained were used to standardize the samples gross energy obtained as a correction factor. The bomb calorimeter was sealed with pure oxygen and the gross energy was directly measured in MJ/kg. The values were obtained and used to calculate for the apparent metabolizable energy (AME) of each treatment diet and faeces using Equation 3.2, as described by Scott & Boldaji (1997).

6.2.5.7 Coefficient of total tract digestibility

The coefficient of total tract digestibility of each nutrient was calculated using the following Equations 61.3-6.5:

Equation 6.1 Apparent metabolizable energy (AME)

$$\text{AME} = \text{Gross energy}_{\text{diet}} - \left[\text{Gross energy}_{\text{excreta}} \times \left(\frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{excreta}}}\right)\right]$$

Equation 6.2 Nutrients consumed (NC)

$$NC\ (g/trial) = Nutrient_{\ analysed\ in\ feed} \times DM_{\ intake}\ (g/trial)$$

Equation 6.3 Nutrients excreted (NEx)

$$NEx=\ (g/trial) = Nutrient_{\ analysed\ in\ excreta} \times DM_{\ excreta}\ (g/trial)$$

Equation 6.4 Digested nutrients (DN)

$$DN\ (g/trial) = Nutrient_{\ consumed} - \left[Nutrient_{\ excreta} \times \left(\frac{Marker_{\ diet}}{Marker_{\ excreta}}\right)\right]$$

Equation 6.5 Coefficient of total tract digestibility (CTTD)

$$CTTD=\ (g/kg) = \frac{Digested\ nutrient}{Nutrient\ consumed}\ .$$

6.2.6 Statistical analysis

The statistical analysis was done using statistical analysis software (Statistica, version 13). One-way ANOVA's were conducted to compare treatments in separate analyses per time point. Normal probability plots were investigated to check for deviations from normality, and were in all cases found to be acceptable. Levene's test was done to test for homogeneity of variance assumptions, and all found to be acceptable. For post hoc testing, Fisher Least Significant Difference (LSD) testing was done.

6.3 Results and discussion

The analysed chemical composition of the five different treatments for the analysed nutrients is shown in Table 6.3. The apparent coefficients of the total tract digestibility (CTTD) of the analysed nutrients and the apparent metabolizable energy (AME) for the five different treatments are shown in Table 6.4. During the first two weeks of a chick's life, their small intestine undergoes many morphological and biochemical changes (Yegani & Korver, 2008), therefore its essential that the diet provided to broilers is able to be utilized efficiently so that the needed energy and nutrients can be used for growth. Importantly, diets that contain fats that are high in saturated fatty acids (SFA), for example the lack of ability of broilers to utilized tallow effectively can lead to digestive and absorption problems in young chicks (UFA) (Azman *et al.*, 2004). In the current study, the lack of differences in the crude lipid (AH) between LO and SF shows that young broilers were able to digest LO as efficiently as SF. This argument is further supported by the lack of differences in AH found between LO with EEP, LES and LYS. In diets of containing low lipid levels (low energy), the supplementation of an emulsifier improved lipid digestibility (Cho *et al.*, 2012) but in the case of the current study, the inability of EEP, LES and LYS to improve the AH of LO shows that young broilers already had the ability to digest LO and is in agreement with the findings of Roy *et al.* (2010) and Jansen *et al.* (2015) who found that the supplementation of an emulsifier had no effect on crude lipid digestibility.Furthermore, the low effect of an emulsifier on lipid digestibility has been associated with the use of fat sources high in

unsaturated fatty acids (Tan *et al.*, 2016 as cited by Huyghebaert G, 2003) as well as other factors such as fat composition, fat physical form and emulsifier type (Patra & Samanta, 2015).

In the study of Kijparkorn (2007), the provision of a low energy diet with an emulsifier improved the protein efficiency of broilers when compared to broilers receiving only the low energy diet. A lack of sufficient energy can lead to protein catabolism in order to make up for the energy needed by broilers resulting in a decrease in the amount of protein to be used for muscle growth (Dabbou *et al.*, 2019). The lack of differences in crude protein (CP) found between LO with SF and with LO with EEP, LES and LYS demonstrates the ability of LO to provide for efficient energy. In chapter 3, the lack of differences in the protein efficiency factor (PEF) and in the growth rate between LO with SF and between LO with EEP, LES and LYS further supports the ability of LO to provide efficient energy during crude lipid digestibility. However, differences in the AME were observed between the different treatments. In Table 6.3, its shows that LO at 10% had a higher AME (18.11 MJ/Kg) than SF at 10% (17.33 MJ/Kg) which may be responsible for the higher AME seen between LO and SF. Significant differences were also found between LO with EEP and between SF with LES and LYS. Various literature have reported an improvement in AME values with the use of an emulsifier (Luc *et al.*, 2010; Roy *et al.*, 2010; Zhang *et al.*, 2011; Kaczmarek *et al.*, 2015). However, both LES and LYS had LO as their main lipid source and did not differ significantly with LO but the significant improvement in the AME seen with LES and LYS when compared to SF could be more due to the ability of broilers to already utilize LO more efficiently than SF rather than due to the ability of LES and LYS to improve the AME.The significant differences in the AME seen between EEP with LO could be due to the interaction between emulsifier and lipid source. This is further supported by the significant differences seen between EEP with LES despite both EEP and LES having LO as the main fat source. In the study of Jones *et al.* (1992), there was an increase in tallow digestibilty with the use of emusifier lecithin and a decrease in tallow digestibility with the use emulsifier lyso-lecithin. Jansen *et al.* (2015) also reported fat type and emulsifier interaction. It was reported that the effect of emulsifier on lipid digestibility were more significant when the fat source was of animal orginal than of plant origin. Therefore, in the current study LO and EEP interaction could have existed contributing to the results obtained for AME in the study. In other words, EEP could have reduced the AME whereas LES and LYS showed no significant effect.

The provision of crude fibre (CF) is essential for the development of the gastrointestinal tract (GIT) of broilers especially for the development and functionality of the gizzard (Mateos & Serrano, 2012). The gizzard plays an important role in controlling various aspects of feed digestion and absorption in the GIT of broilers which include reduction of feed particle size, regulation of the feed motility and flow, controlling of gastroduodenal refluxes and thereby the improvement of the digestion and absorption process of digesta (Mateos & Serrano, 2012). Therefore, dietary fibre influences the passage rate of feed and could have an effect on nutrient absorption in the intestines (Mateos & Serrano, 2012). In the current study, the lack of differences ($P > 0.05$) found between LO and SF shows that LO had no effect on CF digestibility. However, there was a reduction in CF digestibility with the supplementation

with EEP (67%) when compared to LO, whereas improvements in CF digestibility where seen with LES (78%) when compared to LO. There is very limited research on the effects of emulsifiers on CF digestibility and the literature that is available show contrasting results. Kaczmarek *et al.* (2015) reported an improvement in CF digestibility as a result of an emulsifier; whereas, Neto *et al.* (2011) reported no improvement in crude fibre digestibility with an emulsifier. Due to the lack of research on the effects of emulsifiers on fibre digestibility, the findings of the current study can only be explained through speculation. Palmqulst & Jenkins (1980) reported that in ruminants, lipids could negatively affect fibre digestion through the (1) physical coating of the fibre by the lipid source thereby reducing the ability of microbes to digest fibre effectively and (2) a reduction in the amount of cation availability and its effects on microbial function and rumen pH. Kaczmarek *et al.* (2015) suggested that the potential improvement in fibre digestibility in broilers by an emulsifier could be due to the ability of an emulsifier to improve lipid digestibility and possibly reduce the amount of lipid available to physically coat the fibre. In the current study, LO and emulsifier interaction could be the cause for the significant differences seen in fibre digestibility. Emulsifier EEP has the lowest CF value (67%), whereas LES had the highest CF value (78%) despite both having LO as the main fat source. It could be speculated that LES might have potentially reduced the coating of fibre by LO thereby improving fibre digestibility. The reduced fibre digestibility by EEP could be due to emulsifier type and lipid source interaction and is supported by the lower AME of EEP (13.54) when compared to LO (14.10) despite LO being the main lipid source. It could be speculated that the use of EEP with LO could have hindered fibre digestibility contributing to a lower CF digestibility. Therefore, LO could be used in broiler production without having a negative effect on CF digestibility. However, emulsifier type together with LO could have a negative effect on CF digestively but it's important to keep in mind that due to the lack of research on emulsifier effect on fibre digestibility, the understanding of these findings observed in the digestibility of CF between LO with EEP and LES is only through speculation.

Table 6.3 Chemical composition of the analysed nutrients of the five different treatments used in the trial

Parameters	Units	Treatments[1]				
		LO	SF	EEP	LES	LYS
AME	MJ/Kg	18.11	17.33	17.70	17.96	17.94
Crude protein	%	22.81	22.94	22.81	23.34	23.25
Crude fibre	%	2.23	2.67	2.10	2.56	2.42
Crude lipid(AH)	%	11.07	10.24	10.57	10.09	11.20
Ash	%	6.54	6.73	6.83	6.52	6.60

[1] LO: Black soldier fly larvae oil control; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte, Apparent metabolizable energy (AME)

Table 6.4 Mean (± standard error) for coefficient of total intestinal tract digestibility (CTTD) of the five different treatments with the apparent metabolizable energy (AME) in young broilers

Parameters	Units	Treatments[1]					P-value
		LO	SF	EEP	LES	LYS	
AME	**MJ/Kg**	14.10^{a} ± 0.106	13.38^{c} ± 0.072	13.54^{bc} ± 0.068	13.85^{a} ± 0.125	13.79^{ab} ± 0.118	**< 0.01**
Crude protein	**%**	0.99 ± 0.000	0.99 ± 0.000	0.99 ± 0.000	0.99 ± 0.000	0.99 ± 0.000	0.10
Crude fibre	**%**	0.70^{bc} ± 0.022	0.75^{ab} ± 0.017	0.67^{c} ± 0.026	0.78^{a} ± 0.011	0.73^{abc} ± 0.027	**< 0.01**
Crude lipid(AH)	**%**	0.97 ± 0.001	0.97 ± 0.001	0.97 ± 0.002	0.97 ± 0.002	0.97 ± 0.002	0.32
Ash	**%**	0.83 ± 0.011	0.85 ± 0.004	0.84 ± 0.007	0.84 ± .0.011	0.84 ± 0.005	0.57

(^{a,b}): Means with different superscripts within the same row differ significantly (P<0.05);[1]LO: Black soldier fly larvae oil control; SF: Sunflower oil control; EEP: 250g/ton Excential Energy Plus; LES: 0.2L/ton Lesitol; LYS: 250g/ton Lysoforte, Apparent metabolizable energy (AME)

Minerals play an important role in various biological processes and can include, protein digestion, alcohol metabolism, electron transfer, osmotic control of water and acid-base balance. Furthermore, mineral deficiencies can include poor feed utilization, poor feed intake and poor growth in broilers (McDonald *et al.*, 2011). Lipid source and its fatty acid composition can have an effect on mineral uptake by broilers. For instance, fats high in palmitic and stearic acid (poorly digested by young broilers) resulted in a decrease in energy utilisation, crude lipid and in calcium uptake by broilers (Atteh & Leeson, 1983). In the current study, the use of LO had no effect on mineral uptake by broiler when compared to SF and is further seen in the lack of differences found between EEP, LES and LYS. The lack of effect (P> 0.05) on mineral uptake by an emulsifier is in agreement with the findings of Roy *et al.* (2010). Therefore, none of the emulsifiers used in the study had an effect on mineral uptake. The lack of differences found with the use of LO when compared to SF in feed intake, growth, FCR and the in crude lipid digestibility without the use of an emulsifier shows that LO had no negative effect on mineral uptake and can support for efficient mineral uptake without an emulsifier on the same level as SF.

6.5 Conclusion

The supplementation of LO and emulsifiers EEP, LES and LYS in the diet of broilers had no effect (positive or negative) on crude protein, crude lipid and ash digestibility when compared to SF. When compared to LO, emulsifiers LES and LYS had no effect on AME; however, emulsifier EEP had a negative effect on the AME and could be attributed to emulsifier and lipid type interaction. LO performed better than SF in the AME and therefore could provide broilers with higher AME than SF. Emulsifier LES improved crude fibre digestibility, whereas EEP was least effective at improving crude fibre digestibility when compared to LO. No differences in the CTTD of crude fibre between LO and SF could indicate that LO could replace SF in broiler production without having a negative effect on CTTD of crude fibre.

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General conclusion

The study was conducted to evaluate whether the supplementation of emulsifiers EEP, LES and LYS, could improve LO utilization and if LO could perform better than SF. Results from the production study showed that none of the emulsifiers and the use of LO had an effect on the growth rate, ADG, FCR, weekly feed intake, PEF, liveability and EPEF of young broilers. The results could suggest that broilers were able to utilise LO efficiently to meet their energy needs for growth without the needed help by an emulsifier and that LO could replace SF in broiler production without having a negative effect on broiler production. Lack of differences in the broiler carcass yields (dressing percentage, breast component) and on the physical meat quality (pH of the breast and thigh and on breast colour) of broilers suggests that the supplementation of EEP, LES, LYS and the use of LO as a fat source had no negative effect on the carcass quality of broilers. This is very important due to the fact that consumers' perception of poultry meat is highly influenced by appearance (colour), texture, juiciness and flavour. However, the use of an emulsifier can increase xanthophyll absorption thereby increasing b* of the thigh which may affect consumer perception of the meat.

Results from the organ and intestinal study suggest that none of the emulsifiers and the use of LO had a negative effect on organ weights and organ to body percentage of the gizzard, liver, spleen and heart of broilers. Importantly, the use of an emulsifier could lead to an immunosuppressive effect on the spleen and could lead to health problems. None of the emulsifiers led to an immunosuppressive effect on the spleen. The use of LO, however had an effect on the size of the bursa of Fabricius. However, the size of the bursa of Fabricus for LO still fell within range that is considered as normal and that the difference is more correlated to final body weight and individual variation and possibly to an improvement in the production immune proteins. None of the emulsifiers and the use of LO had a negative effect on the pH of the duodenum, ileum and caeca. However, LES had an effect on gizzard and jejunum pH which may be related to its possible effects on digest retention time in the gizzard causing a decrease in gizzard pH. This in turn could influence the amount of duodenal secretions needed to buffer against the acidic content (increase in pH) when it enters the intestines. Despite these differences, the pH of the gizzard and jejunum for treatment LES still fell within the normal pH range for both these organs. The lack of differences found in the gizzard score in the primary and in the gizzard trial suggests that none of the emulsifiers (at standard and double the standard level) and the use of LO contained any substance that could have negatively affected the quality of the gizzard. The significant differences found in the L* coordinate of the liver between LO with emulsifiers EEP and LES could be due to the effect of LO and emulsifiers on lipid accumulation, however tmore research is required. In the last chapter digestibility study, none of the emulsifiers and the use of LO had an effect on the CTTD of crude protein, crude lipid and ash. This could suggest that the use of EEP, LES, LYS and LO had no negative effect on the utilization of crude protein, crude lipid and ash by broilers. EEP showed to have had a negative effect on the CTTD of the AME of broilers; whereas LES and LYS had no effect on the CTTD of AME. This could suggest that LO is able to be utilized efficiently by broilers without the help of an emulsifier and performed better than SF and therefore could improve the performance of broilers. In terms of crude fibre, LES was the most effective (highest CF value) at

improving CF; whereas EEP was the least effective (lowest CF value) and LYS showing slight improvement in CF when compared to LO. This could suggest that emulsifier type could affect crude fibre utilization. Furthermore, the lack of differences found between LO and SF suggest that LO could be utilised in broiler production without having a negative effect on crude fibre utilization.

Overall, the use of emulsifiers and its effects on the various parameters of broiler performance have shown varying results on organ an intestinal pH, on thigh colour, CTTD of crude fibre and AME. Data published on emulsifiers utilised commercial oils which include palm oil, sunflower oil, tallow poultry lipid and soybean oil. There is a lack of research on the effects of emulsifiers with LO. The current results of the study show that young broilers were able to utilise LO efficiently on the same level as SF without an emulsifier and showed limited negative effect on broiler production, meat quality traits, gizzard health and on nutrient utilization. This indicates that LO could be seen as a promising alternative to sunflower oil in broiler production. On the other hand, the ability of emulsifiers is not only limited to the digestion of lipid and has shown to have an effect on meat colour, intestinal pH, nutrient utilization of which most are not influenced by lipid type but more on emulsifier functionality. Therefore, further research is recommended to investigate the use of emulsifiers on the various sections responsible for feed digestion and absorption in young broilers of which include gizzard functionality, possible changes in intestinal morphology affecting the uptake of various nutrients and its effects on liver functionality during different stages of broiler growth.